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CONTRIBUTIONS TO THE GENETICS OF DROSOPHILA MELANOGASTER.

I. THE ORIGIN OF GYNANDROMORPHS.

By T. H. Morgan and C. B. Bridges.

II. THE SECOND CHROMOSOME GROUP OF MUTANT CHARACTERS.

By C. B. Bridges and T. H. Morgan.

III. INHERITED LINKAGE VARIATIONS IN THE SECOND CHROMOSOME.

By A. H. Sturtevant.

IV. A DEMONSTRATION OF GENES MODIFYING THE CHARACTER "NOTCH."

By T. H. Morgan.

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CONTENTS.

PAGE.

I. *The Origin of Gynandromorphs.* By T. H. MORGAN and C. R. BRIDGES..... 1
Frequency of Occurrence of Gynandromorphs..... 10
Relative Frequency of Elimination of the Maternal and Paternal Sex Chromosomes..... 11
Distribution of Segmentation Nuclei as deduced from Distribution of the Characters of Gynandromorphs..... 12
Starting as a Male *vs.* Starting as a Female..... 12
Cytological Evidence of Chromosomal Elimination..... 13
Earlier Hypotheses to explain Gynandromorphs..... 17
The Origin of the Germ-cells in Flies..... 22
Courtship of Gynandromorphs..... 22
Phototropism in Mosaics with one White and one Red Eye..... 23
Sex-limited Mosaics..... 24
Somatic Mosaics..... 26
Somatic Mutation..... 27
Mosaics in Plants..... 32
Classification and description of Gynandromorphs of *Drosophila*..... 33
Gynandromorphs approximately bilateral..... 35
Gynandromorphs mainly female..... 41
Gynandromorphs mainly male..... 48
Gynandromorphs roughly "fore-and-aft"..... 51
Gynandromorphs produced by XXY females..... 53
Gynandromorphs of complex type..... 55
Special Cases..... 57
Gynandromorphs with incomplete data..... 70
Drosophila Gynandromophs previously published..... 72
Gynandromorphs and Mosaics in Bees..... 74
Gynandromorphs in Lepidoptera..... 81
Other Insects..... 94
Spiders..... 94
Crustacea..... 95
Molluscs..... 97
Echinoderms..... 98
Vertebrates..... 98
Fishes..... 98
Amphibia..... 99
Reptiles..... 101
Birds..... 101
Mammals—Man..... 106
Is Cancer a Somatic Mosaic?..... 108
Is the Freemartin a Gynandromorph?..... 109
Summary..... 111
Literature Cited..... 116

II. *The Second Chromosome Group of Mutant Characters.* By C. B. BRIDGES and T. H. MORGAN..... 123
Introduction..... 125
Chronological list of the II Chromosome Mutations..... 126
Map of Chromosome II..... 127
Speck..... 128
Olive..... 135
Truncate..... 136
Truncate Lethal..... 138
Snub..... 140
Truncate Intensification by Cut..... 143

PAGE.

II. *The Second Chromosome Group of Mutant Characters*—continued.
Black ... 144
Balloon ... 148
Vestigial ... 150
Blistered ... 155
The Semi-Dominance of Blistered—Free-Vein ... 158
Jaunty ... 160
A Mutating Period for Jaunty ... 161
Curved ... 164
Purple ... 169
The Differentiation of Purple by Vermilion—Disproportional Modification. 170
No Crossing Over in the Male ... 174
The Inviability of Vestigial—Prematuration, Repugnance, Lethals ... 177
The Purple "Epidemic"—"Mutating Periods" ... 178
Balanced Inviability, Complementary Crosses ... 181
The Variation of Crossing Over with Age ... 183
Coincidence ... 188
The Relation between Coincidence and Map Distance ... 188
Special Problems Involving Purple—Age-Variations, Coincidence, Temperature-Variations, Cross-Over Mutations, Progeny Test for Crossing-Over 193
Strap ... 200
Arc ... 202
Gap ... 208
Antlered ... 211
Dachs ... 216
Streak ... 222
Dominance and Lethal Effect of Streak, Parallel to Yellow Mouse ... 223
Comma ... 228
Morula ... 230
Female Sterility of Morula ... 230
Apterous ... 236
$C_{II\,l}$ and $C_{II\,r}$ ... 239
Cream II ... 239
Trefoil ... 244
Cream b ... 245
Pinkish ... 247
The Double-Mating Method ... 248
Plexus ... 251
Limited ... 254
Confluent ... 255
Confluent *Virilis* ... 257
Fringed ... 257
Star ... 259
Lethal Nature of the Homozygous Star ... 260
Crossing Over in the Male ... 263
Nick ... 273
Vestigial-Nick Compound ... 275
Dachs-Lethal ... 277
Dachs-Deficiency? ... 278
Balanced Lethals ... 279
Squat ... 283
Lethal II*a* ... 286
Telescope ... 291
Second Chromosome Modifiers for Dichæte Bristle Number ... 293
Dachsoid ... 294
The Construction of the Map of the Second Chromosome ... 297
Summary of Available Data on Crossing Over in the Second Chromosome ... 298
Constructional Map ... 302
Working and Valuation Map ... 303
Bibliography ... 304

PAGE.

III. *Inherited Linkage Variations in the Second Chromosome.* By A. H. STURTEVANT. 305
Introduction 307
"Nova Scotia" Chromosome 307
Tests of Cross-Overs 312
Right-hand end of Nova Scotia Chromosome ($C_{II\,r}$) 313
Left-hand end of Nova Scotia Chromosome ($C_{II\,l}$) 316
Homozygous $C_{II\,r}$ 319
With $C_{II\,l}$ 319
Without C_{II} 321
No Tests of Homozygous $C_{II\,l}$ 322
Tests Showing No Crossing-Over in Males 322
Constitution of the Nova Scotia Stock 322
Another Second-Chromosome Linkage Variation 324
Comparison with Results obtained from C_{III} 325
Discussion 327
Summary 330
Appendix 331
Literature Cited 341

IV. *A Demonstration of Genes Modifying the Character "Notch."* By T. H. MORGAN 343
Variation of Notch 346
The Problem 347
Condition of Stock before Selection 348
Selection of Females having Notch in one Wing only 349
Selection of Somatically Normal Winged Females that are Genetically Notched Females 350
Duplicate Selection Experiment 355
Localization of the Gene for Notch 358
The Indentification of the Modifying Genes 361
Short Notch 364
First Test 366
Second Test 367
Third Test 368
Fourth Test (for fourth-chromosome modifiers) 369
Recombination of Bent and Short Notch 370
Crosses between Short Notch and Other Stocks 371
Short Notch by Star Dichæte 372
Short Notch by Eosin Ruby Forked 372
Classification of Types of Notch 376
Aberrant Notch Wings 379
Deformed Eyes 379
Little Eyes 381
High Sex-ratios Caused by Lethals 381
Other Characters that Look Something Like Notch 382
Gynandromorph; Notch Eosin Ruby 384
Summary 387

I.

THE ORIGIN OF GYNANDROMORPHS.

By T. H. Morgan and C. B. Bridges.

With four plates and seventy text-figures.

I. THE ORIGIN OF GYNANDROMORPHS.

By T. H. Morgan and C. B. Bridges.

INTRODUCTION AND GENERAL DISCUSSION.

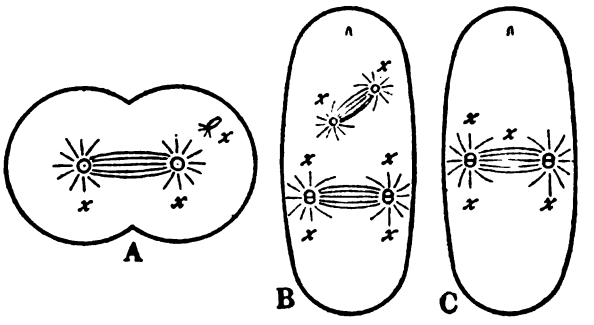
The sharp distinction into two kinds of individuals, males and females, characteristic of so many animals, is occasionally done away with when an individual appears that bears the structures peculiar to the male in some parts and to the female in other parts of the body. Such an individual may show not only the secondary sexual differences (either sex-limited or sex-linked) of male and female, but gonads and genitalia of both kinds as well. We speak of these as gynandromorphs. The union of the two sexes in a single individual shows how far the characteristics normally associated with one sex alone are compatible with the presence in another part of the same body of somatic structures and reproductive organs of the opposite sex. In a word, how far each is independent of sex hormones. But the chief importance of these rare combinations lies in the opportunity they furnish for analysis of the changes in the hereditary mechanism of sex determination that makes such combinations possible. This evidence is chiefly derived from gynandromorphs that are also hybrids. Such individuals may combine not only male and female sex differences, but the characteristic racial differences as well. Whether gynandromorphs arise more frequently in hybrids or whether it is only that their detection is easier under such circumstances will be discussed later. The occurrence of hybrid gynandromorphs offers at any rate a unique opportunity to discover the method of origin of such kinds of individuals.

In hybrid gynandromorphs the differences that are shown may be due to genes carried by the sex chromosomes. Most of the gynandromorphs of *Drosophila* belong to this category. In many cases, however, especially in other insects, it is not known whether the differences shown by the hybrid gynandromorph are due to the sex chromosomes or to other chromosomes, either because the ancestry of the gynandromorph is unknown or because the method of inheritance of the gene is unknown. There are, however, some very rare cases in *Drosophila* in which the characters involved are probably autosomal and the individual, while showing its dual parentage in different parts of the body, is not a sex-mosaic. It may be convenient to designate such types as mosaics, while the sex-mosaics may be designated by the more special term gynandromorphs.

In our work on *Drosophila melanogaster* (*ampelophila*) a large number of gynandromorphs and mosaics have appeared, and since the first

description of a few of them was published we have continued to keep records of their occurrence. Others, too, working with our mutant types have found them, and a few have been described by Dexter, Duncan, and Hyde. We soon realized that they occurred with sufficient frequency to make it possible to devise experiments of a sort to furnish the long-sought criterion as to the most common method of their occurrence. It is this evidence on which we wish now to lay chief emphasis.

The ordinary gynandromorph is an animal that is male on one side of the body and female on the other. The reproductive organs, gonads, and ducts may or, in bees at least, may not show a corresponding difference. A typical case of a gynandromorph that is bilateral, at least superficially, is represented in plate 1, figure 1. For a long time it has been recognized that bilateral gynandromorphism is only one kind of abnormal distribution of the sex characters; even in the classical case of the Eugster bees (see p. 74) other distributions of the characters were recorded. In the fly represented in plate 3, figure 2, the upper part of the abdomen is female, but the lower side of the abdomen, notably the external genitalia, are male. In the individual represented in plate 3, figure 5, the left anterior side of the head is male, the right female, while the left posterior parts of the body are female, the right male. Other cases will be described later in which even more irregular and complex distributions of male and female parts exist.



TEXT-FIGURE 1.

Before discussing these and other cases in detail, it may be well to give three of the most recent interpretations of gynandromorphism resting on a chromosomal basis and the criteria by which the validity of each has been tested.

In 1888 Boveri suggested that on rare occasions a spermatozoon, on entering the egg, might be delayed in its penetration to the vicinity of the egg-nucleus, and the latter might meanwhile have begun to divide, so that the sperm-nucleus came to unite with only one of its halves. In consequence, two kinds of nuclei would be produced in the embryo (text fig. 1 A). The nuclei that come from the sperm plus the half egg-nucleus would be diploid. If, as in the bee, one nucleus stands for the male and two for the female, it follows in such cases that all those parts of the body whose nuclei are derived from the

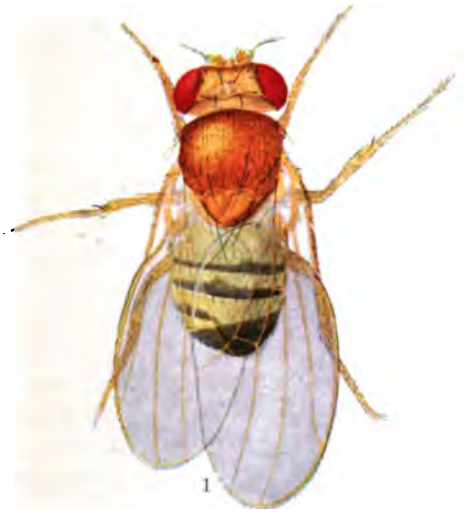
single (haploid) nucleus would be male, all those from the double (diploid) would be female. Moreover, if the two differ in one or more characters, the male parts of the gynandromorph should be expected to be like the mother, *i. e.*, maternal, and the female parts should be paternal if the paternal characters involved are dominant. The possibility of testing Boveri's hypothesis was pointed out by one of us (Morgan) in 1905, and a test case was apparently furnished by a hybrid gynandromorph of the silkworm moth described by Toyama. The result was not in harmony with Boveri's hypothesis, but since the relation of one or of two nuclei to sex was not then known for moths, the case is not decisive, as will be shown more at length later. On the other hand, Boveri's discovery of some preserved specimens of the original Eugster gynandromorph bees and his analysis of their hybrid characters seemed to show that the condition of these bees was compatible with his theory. This evidence will also be taken up more fully later. We may anticipate our account of hybrid gynandromorphs of *Drosophila* and state that they furnish direct evidence against Boveri's hypothesis, for these flies at least.

In 1905 Morgan suggested an alternative hypothesis based on the fact that more than one spermatozoon had been found to enter the bee's eggs. Should one only of these sperm-nuclei unite with the egg-nucleus, the combination would give rise to the diploid cells of the embryo, while if a second (or a third, etc.) sperm-nucleus should develop it would give rise to haploid cells in the rest of the embryo (fig. 1 B). On this view the haploid cells should be paternal and produce male parts, and the diploid cells maternal and produce female parts, which is exactly the reverse relation in regard to parental origin of the male and female parts from that expected on Boveri's hypothesis. A decision as to which view is correct might be reached in any special case in which sex-linked characters enter from the paternal and maternal sides. As will be shown later, some of the evidence from the *Drosophila* gynandromorphs is incompatible with this hypothesis of Morgan.

A third hypothesis that grew out of the work done in this laboratory was published in 1914 by Morgan, based on evidence from the *Drosophila* cases. On this view the gynandromorphs are due to an *elimination of one of X chromosomes*, usually at some early division of the segmentation-nuclei. Rarely, in consequence of a delay in the division of one of the X chromosomes, one of the daughter-halves fails to reach its pole and is lost in the mid-plate or in the cell-wall (fig. 1 c). As a result, the embryo comes to carry two kinds of nuclei, one kind containing one X and the other kind two X chromosomes. The critical evidence in favor of this interpretation is found in the presence on both sides of the gynandromorph of other mutant characters whose genes are not in the X chromosomes, but in autosomes. If, for example,

the mother contains a mutant gene in one of her autosomes and the father contains its normal allelomorph, it is expected, on Boveri's view, that the male side of the gynandromorph should show this maternal autosomal character, even though recessive. But on the hypothesis of chromosomal elimination, both sides of the gynandromorph should show the same autosomal characters. Conversely, if the cross is so arranged that a recessive mutant autosomal gene enters from the father's side, then, on Morgan's earlier view of polyspermic fertilization, the male side of the gynandromorph should show this recessive mutant character; but on the elimination hypothesis both sides should show the same (dominant) autosomal characters. It may now be shown by critical examples that the hypothesis of chromosomal elimination will cover nearly all of the cases of *Drosophila*, and is therefore preferable to either of the other two, even although in special cases either of these two other ways of producing gynandromorphs may be realized. A few additional cases have been found that call for still other interpretations.

The critical cases are as follows: A yellow white male was mated to a female pure for the recessive autosomal genes for peach eye-color, spineless body, kidney eye-shape, sooty body-color, and rough eyes. A gynandromorph was found (plate 1, fig. 1) that was male on one side, as shown by his shorter wing, sex-comb on the foreleg, and the shorter bristles characteristic of the male (the body was also slightly bent to the smaller male side), and female on the other side, as shown by the converse characters to those just given. The gynandromorph possesses on both sides all of the characters dominant to the five recessive autosomal factors that came in with the spermatozoon. On Boveri's explanation, the male side should have a yellow body-color and a white eye, because their two genes are carried by the maternal nucleus, while the female side should show the normal characters of the wild fly, as is the case. The absence of yellow body-color and white eyes on the male side rules out his explanation. On Morgan's hypothesis of polyspermy, the male side that comes from one or more supernumerary sperms should show the five autosomal recessive characters brought in by each sperm, which is not the case, and the female side should show the normal characters, as it does. The absence of the five recessive characters on the male side rules out this explanation also. On the theory of chromosomal elimination the gynandromorph started as an ordinary XX female—one X carrying the genes for yellow and for white, the other carrying their normal allelomorphs, viz, genes for gray and for red. Either of these chromosomes might be the one to be eliminated, *i. e.*, at some division either one of the yellow white daughter chromosomes failed to reach one of the daughter cells, or one of the gray red daughter chromosomes failed. If the former, the male side should get only the gray



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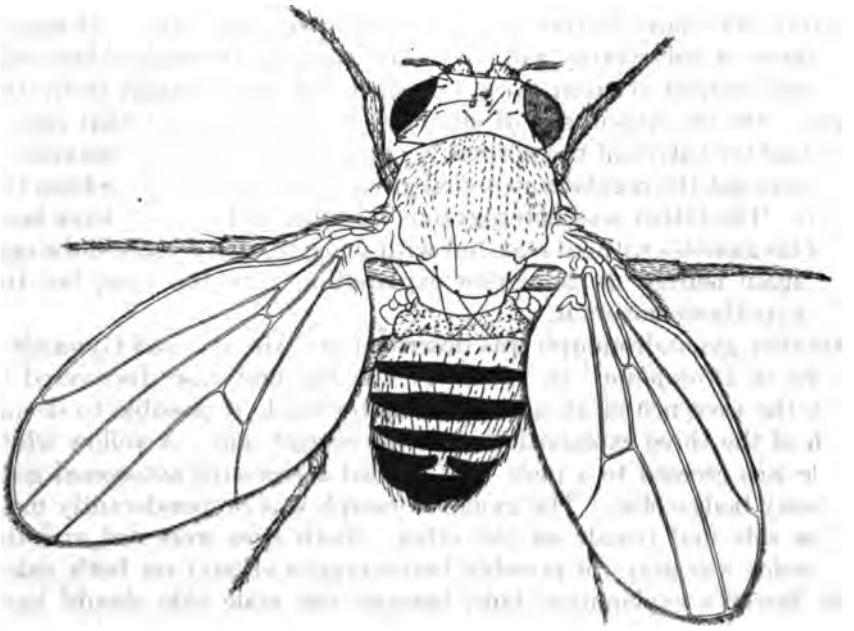
E. M. Wallace Pinx

GYNANDROMORPHS OF DROSOPHILA

red chromosome, and show the corresponding characters, which in fact it does. If the other chromosome had lost one of its halves at the critical division, the male side should be yellow white, which is not the case. Evidently, then, it must have been a yellow white daughter chromosome that was lost in this case. In regard to the five autosomal characters, it is clear that since both male and female sides show all the dominant characters, both sides of the body received the autosome that bears their genes. This hypothesis thus covers the facts in the case. Sections of the abdomen showed abnormal gonads that appeared to be testes.

Another gynandromorph is drawn in plate 1, figure 2. It, too, came from this same cross of a yellow white male by a female of a race with the same five recessive characters. It is not a bilateral



TEXT-FIGURE 2.

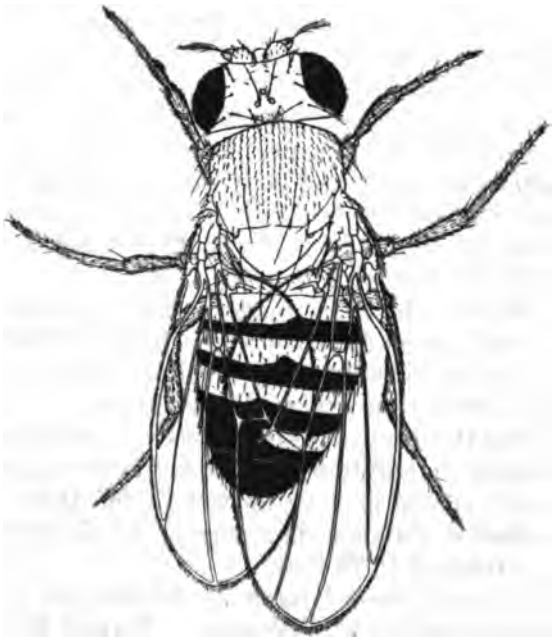
gynandromorph, but more nearly an anterior-posterior combination. The abdomen is male, and since the forelegs bear no sex-combs, some at least of the anterior end is female. One wing is male; at least it is shorter than the one on the opposite side, which is presumably female. As in the last case, the fly shows only the characteristics belonging to the normal allelomorphs of the five recessive autosomal factors. The analysis here is the same as above.

Another gynandromorph, drawn in text-figure 2, arose from a cross between a male that was heterozygous for the two dominant autosomal genes for star eyes and for dichæte bristles and a female that was notch

("short" type). The mother had one sex chromosome with the dominant gene for notch and another sex chromosome that had the normal allelomorph of notch and also a gene for eosin eye-color. The gynandromorph was male on one side, with an eosin eye (with a red fleck in it), a sex-comb, and a short wing on that side, and female on the other side with a red eye, no sex-comb, and a longer wing. The genitalia were male. The gynandromorph arose by the fertilization of an egg containing the sex chromosome bearing the eosin eye-color (because had the other maternal X chromosome been present one of the wings, or both, would have shown the notch character). In this case it was the X chromosome from the father that was eliminated, since the male side shows the eosin eye-color of the maternal sex chromosome. Boveri's explanation will not fit this case, even though the male side shows a maternal character, viz, eosin eye, because that side is dichæte, hence contains dominant factors from the paternal autosome. Morgan's hypothesis of polyspermy will not fit this case, for the male side should have red instead of eosin eye-color, since red was brought in by the sperm. On the hypothesis of elimination, it is apparent that one of the daughter halves of the normal X chromosome was lost; the cells of both sides got the regular autosomal groups, for dichæte came from the father. The father was heterogyzous for star, and it must have been one of his gametes without star, but with dichæte, that fertilized the egg. Here again neither of the earlier explanations fits the case, but the third hypothesis covers it.

Another gynandromorph was described in "Mosaics and Gynandromorphs in *Drosophila*" in 1914. It was the first case discovered in which the presence of an autosomal factor made it possible to decide which of the three explanations was the correct one. A yellow white female was crossed to a male that carried a recessive autosomal gene for ebony body-color. The gynandromorph was preponderantly male on one side and female on the other. Both eyes were red and the body-color was gray (or possibly heterozygous ebony) on both sides. Here Boveri's explanation fails, because the male side should have been entirely maternal, therefore yellow and white; and Morgan's earlier explanation fails, because the male side was not ebony. On the elimination hypothesis a maternal yellow white daughter chromosome was lost; hence both sides had red eyes and not yellow body-color, and both sides received the same normal autosomes. This cross, in which a yellow white female was mated to an ebony male, was carried out extensively (January to May 1914) and 6 more gynandromorphs were found. However, in order to discriminate between partial fertilization and polyspermy on the one hand and elimination on the other only those cases are diagnostic in which the male parts come from the father and show at the same time autosomal parts from the mother.

Another gynandromorph (obtained by Sturtevant), text-figure 3, came from a mother that had in one second chromosome the genes for C_{ul} and for curved, and in the other the genes for black and for vestigial. She may have had a third chromosome gene for crossing-over. The father was homozygous for black, purple, curved, plexus, speck, all in the second chromosome. Brothers and sisters were as expected; the black curved crossing over was 28 per cent. The fly was black and showed no trace of purple, vestigial, curved, plexus, or speck. It was male on the left side, female on the right, except for head bristles. The genitalia were male. The fly was sterile. Unless the egg were a double cross-over for black vestigial curved, which is unlikely, it contained a black vestigial bearing chromosome. The sperm contained the five second-chromosome genes. Since the male parts showed none of these second-chromosome characters, except black, although all the rest except purple might have been visible, it is highly probable that the male parts contained both second-chromosomes. The result shows at least that the theory of chromosome elimination is a more probable explanation than partial fertilization or multiple fertilization, and the result would be conclusive if the possibility of double crossing-over were rejected.



TEXT-FIGURE 3.

Another case (found by Sturtevant, 4079 C, Oct. 31, 1917) occurred in a cross betweem a male with a normal X chromosome and pure for the second-chromosomal genes for black, purple, and curved, and a forked female that was heterozygous for the second-chromosomal genes for black, purple, and curved. The gynandromorph (plate 1, fig. 3) had a short wing on the left side, but the left foreleg was not male. The abdomen had the male banding and genitalia and contained two testes. No forked bristles were found in any part of the body. Elimination of one of the forked-bearing maternal X chromosomes left the wild-type X chromosomes to determine the character of the male parts.

The gynandromorph must have received the normal second chromosome from its mother (since normal autosomal characters only appeared) and a second chromosome from its father with the three recessive genes. Since neither male nor female parts show these recessive genes, two second chromosomes must have been present in all the nuclei, both in the male and in the female parts.

FREQUENCY OF OCCURRENCE OF GYNANDROMORPHS.

In general, we have no record of the frequency of the occurrence of gynandromorphs. They are found from week to week, their number being roughly in proportion to the number of flies passing under observation, and also in proportion to the care with which the flies are scrutinized in detail. On four occasions, however, the frequency of their appearance was recorded.

In the first case (in 1914) a cross, involving yellow flies, white-eyed and eosin-eyed flies, and wild-type flies, seemed to give gynandromorphs more often than usual. It is to be noticed that the striking color differences of eye and body in this combination would, as a rule, make it easy to detect hybrid gynandromorphs, and their frequency may have been due to this fact. In all 32 gynandromorphs were found in a total of 42,409 flies, or 1 in 1,325.

Duncan, in 1915, made a careful examination of hybrid flies and found 3 gynandromorphs in a total of 16,637 flies, or 1 in 5,500. All flies were so thoroughly scrutinized that probably most of the gynandromorphs that occurred were found.

The third set of observations was made on material that was chosen because, in addition to sex-linked factors, autosomal genes were present, which should give an answer to the three contrasted hypotheses described in the preceding pages. In all, 2 gynandromorphs were found in a total of 4,979 flies.

A fourth record made by Sturtevant also involved autosomal as well as sex-linked characters. Forked females were mated to males with normal bristles. The female was heterozygous for the second-chromosome genes, black, purple, curved; the male homozygous for the same genes; 3 gynandromorphs were found in about 24,000 offspring.

Taking all these results together, the observed ratio is 1 gynandromorph in 2,200 flies.

Whenever the chromosomal elimination occurs at an early stage in development, or when the color or structural difference involved is striking, the gynandromorph is more likely to be found than when the contrary conditions are present. If elimination occurs late in development the region affected may be so small as to escape detection. It seems probable, therefore, that such irregularities may be more frequent than the figures given above indicate.

It is a curious fact that practically all of the mosaics of *Drosophila* involve the sex chromosomes. It is true that the differences in the sexes are so marked that individuals partly male, partly female, could easily be detected on this basis alone. On the other hand, the mutant characters that are sex-linked are not more striking than are those of autosomal mutants. The almost complete absence of the latter kind of mosaics in our cultures shows very positively that elimination is very infrequent in these chromosomes, or, if it occurs, that an individual or part with only one autosome is less likely to survive than an individual with one X chromosome. Until this question is settled it can not safely be concluded that the sex chromosomes suffer elimination more than do the autosomes. The fact that autosomal non-disjunction has not yet been observed in *Drosophila*, though looked for, lends support to the view that variations in autosomal number are either rare or are fatal.

RELATIVE FREQUENCY OF ELIMINATION OF THE MATERNAL AND PATERNAL SEX CHROMOSOME.

It might have been supposed *a priori* that delay in the unraveling of the chromosomes of the sperm might be the most frequent cause of the elimination of chromosomes. As a matter of fact, the evidence shows clearly that the maternal X is as likely to be eliminated as the paternal. For example, we find on looking through our records that in 15 cases the maternal X chromosome and in 15 cases the paternal chromosome must have been the one eliminated. There were 16 cases in which from the nature of the cross or of the result it could not be determined which one was eliminated. In the above estimation we also have left out of account all cases that were entirely male, or for which special explanations are called for. There can then be no doubt but that elimination is somehow connected with the nature of the X chromosomes themselves, such as slowness in dividing or in reaching the poles of the spindle, and that elimination is not due to delay in the development of either pronucleus.

An examination of the gonads in *Drosophila* gynandromorphs has shown in every case that the two gonads are the same, *i. e.*, both are ovaries or both are testes. Even in bilateral types the two gonads are alike. Duncan found this true for the few cases that he sectioned. This number was, however, insufficient to establish the rule, but we can now add about 20 other cases to the list. There can remain no doubt that the gonads are alike, regardless of the way in which the male and female parts are distributed on the surface. The results are in accord with the early formation of the germ-cells in Diptera and probably mean that both gonads are derived from one and the same cleavage nucleus.

DISTRIBUTION OF SEGMENTATION NUCLEI AS DEDUCED FROM DISTRIBUTION OF THE CHARACTERS OF GYNANDROMORPHS.

If the first division of the segmentation nucleus corresponds with the right and left sides of the embryo, and if chromosomal elimination is more common at this time or more easily detected, we should expect most gynandromorphs to be roughly bilateral. We have found that this is the most frequent type. If the first division were in the antero-posterior direction and elimination were frequent at this time, we should expect to find some gynandromorphs with the anterior end of one sex and the posterior end of the other sex. This type also is fairly frequent.

If the first division were dorso-ventral we might expect corresponding gynandromorphs, but, although more difficult to detect, they appear almost never to be of this kind.

If the second division were a time of elimination we would expect quadrants instead of halves. Such cases are known.

The striking fact about the gynandromorphs is that large regions of the body are involved. Granting that later differences would be less easily detected, in certain organs at least, the results are so emphatically in favor of large parts of the body being involved that we think it highly probable that the elimination is most frequent in the first division.

The difficulty of reaching a decision is greatly increased when it is recalled that from the ventral plate of the embryo the serosa is formed by a folding upward of the sides of the plate. How much of the ventral ectoderm is carried in this way to the dorsal surface is not known. Should it replace the dorsal covering derived from the segmentation nuclei (that goes then into the serosa which is later thrown off), the results for ectodermal organs are restricted to the regions on each side of the ventral plate. The mesoderm also grows from the ventral to the dorsal surface, and presumably mesodermal dorsal structures have come from ventral material.

A further complication arises in connection with the imaginal plates out of which many adult organs are produced. Unless the exact origin of their cells is known, it is not possible to safely conclude at what time the early elimination takes place.

STARTING AS A MALE VERSUS STARTING AS A FEMALE.

The evidence recorded in the preceding pages is analyzed on the basis that the gynandromorph starts as an XX individual, or female, and that the male parts arise by the elimination of an X from one of the cells. The evidence from hybrid combinations shows very clearly that practically all of our gynandromorphs have started as XX individuals, as 19 are more female, 14 nearly equal, 6 more male.

There are, however, other theoretical possibilities that should be noticed, for it is possible that gynandromorphs may sometimes arise in other ways. In fact, one or two of those we describe may be explained in the following way: An X egg fertilized by a Y sperm (a regular male), might later become partly female, *i. e.*, gynandromorph, through somatic non-disjunction, both daughter X's remaining in the same cell at some early embryonic division. Parts descended from the XXY cell are female; the other (Y) cell would presumably die. If such a process occurred at the first division and all of the yolk was later occupied by the viable XXY cells, the embryo would become entirely female, although containing only sex-linked genes from the mother, and might be mistaken for a case of 'primary non-disjunction.'

A non-disjunctionally produced egg containing a Y chromosome or an egg without a sex chromosome fertilized by an X sperm might also, starting as a male, produce a purely paternal female or female parts (mosaic) through somatic non-disjunction. If non-disjunction occurred at a late division a proportionately smaller part of female tissue would be formed and the regular male cells formed earlier would give male parts—*i. e.*, the individual might be more male than female.

There are no cases where these explanations only will apply, but a few cases accounted for by chromosome elimination may be also explained in one or the other of these ways, viz, that the gynandromorph started as a male.

CYTOLOGICAL EVIDENCE OF CHROMOSOMAL ELIMINATION.

The most important case of chromosomal elimination involving one of the sex chromosomes, and therefore most like the case of gynandromorphism in *Drosophila*, has been described in *Ascaris* (*Rhabditis*) *nigrovenosus* by Boveri and by Schleip. In this nematode there is a hermaphroditic generation that lives in the lungs of the frog. Eggs and sperm are produced at the same time in the hermaphroditic gonad. The full number of chromosomes is the same in the early oögonia and spermatogonia. This number is reduced to half in the egg and also in the sperm at the reduction division, but while all the eggs are alike, there are two kinds of spermatozoa, one containing one less chromosome than the other. This loss of one of the chromosomes in one-half of the sperm-cells is apparently brought about as a regular process by the failure *at reduction* of one member of the paired sex chromosomes to reach the pole. It is caught at the division plane or else remains near that plane and disappears. This process differs however, from what we suppose to occur in eliminating a sex chromosome in *Drosophila* when a gynandromorph is produced in that an undivided X is lost. Whether in *Ascaris* this process occurs in all the cells at a given division or is somewhat irregular is not certain, and can only be determined by a fuller knowledge of the ratio of males

to females that result. Boveri thought, from the evidence obtained, that the loss of one chromosome at this time is a constant phenomenon. If so, it differs in this regard from the rare occurrence of elimination in *Drosophila*.

In the group of aphids and phylloxerans a process occurs that has at least a certain analogy to elimination. When the male-producing egg, which is smaller (in the latter group) than the female-producing egg, throws off its single polar body, one sex chromosome is eliminated from the egg, although the autosomes divide equationally at this time. This elimination is not due to loss of a daughter chromosome, because it is preceded by a sort of synaptic union and disjunction of the chromosome in question. Here the lagging of one whole chromosome in the middle part of the spindle, and its failure to reach the outer pole in time to become incorporated in the nucleus of the polar body, furnishes a certain resemblance, at least, to the elimination process.

In one species, *P. fallax*, there are four sex chromosomes, two of which are eliminated from the male-producing egg, as described above. There remain, then, two sex chromosomes in the male. When the sperms are produced these two do not act as mates when the other chromosomes (autosomes) pair and segregate, but both pass together to one pole. The daughter cells that get them become the functional female-producing spermatozoa; the other cell that lacks them degenerates. Here, then, although two sex chromosomes are present, they both pass to one pole. This behavior is quite unlike the results produced by chromosomal elimination.

In one of the aphids Morgan found a cyst in which, owing apparently to the failure of the autosomes to pair before segregation, an irregular distribution of the chromosomes took place, including an erratic distribution, somewhat imperfect, it is true, of the sex chromosomes also. This unusual and irregular occurrence might lead to complication in the distribution of the sex chromosomes in the next generation, if such sperm were to become functional, and furnish a parallel case to the phenomenon of primary non-disjunction that Bridges has described in *Drosophila*.

In *Drosophila* there takes place on rare occasions an erratic distribution of the sex chromosomes, either in the male or in the female, that has been called primary non-disjunction. Occasionally, both sex chromosomes are eliminated in the polar body, leaving in the egg the haploid number of chromosomes, but not a sex chromosome. If such an egg is fertilized by a female-producing sperm containing one X chromosome, an XO male results. The male, lacking the characteristic Y chromosome of the normal male, nevertheless resembles a normal male in all respects, except that he is sterile. Conversely, in other cases, both X chromosomes may remain in the egg. Such an egg does not develop if it is fertilized by a female-producing sperm giving it three X's, but

if such an egg is fertilized by a male-producing Y-bearing sperm, it produces a female XXY, that is like a normal female in its somatic characters; but such a female, owing to the presence of three sex chromosomes (XXY), gives rise to the phenomenon of secondary non-disjunction to be described presently.

Similarly in the male, primary non-disjunction may take place in the formation of the spermatozoon. If at the reduction division the X and Y chromosomes, that normally pass to opposite poles, should pass to one pole, a spermatozoon would result from one of the daughter cells that contains both an X and a Y, and such a sperm fertilizing an X-bearing egg would give rise to an XXY female that would exhibit secondary non-disjunction. The other daughter cell without X or Y also produces a functional sperm. In these cases of primary non-disjunction an irregular distribution of the sex chromosomes leads to unusual types of sex-linked inheritance, but not to gynandromorphism or to mosaics.

In secondary non-disjunction, owing to the presence of three sex chromosomes, any two of which may form a pair, there is left one chromosome without a mate. Genetic analysis shows that the unpaired chromosomes, in some cases one of the X's, in others the Y, may either pass out of the egg at maturation or remain in the egg. Aside from this irregularity there is not much in the process that is akin to the kind of chromosomal elimination postulated for gynandromorphs, since the processes underlying the two phenomena are probably quite different. These cases furnish exceptions in regard to genetic behavior and furnish important evidence bearing on the determination of sex, but do not lead to the kinds of effects seen in the production of gynandromorphs, except when the non-disjunction occurs at a cleavage stage, as already explained.

As stated, Boveri based his hypothesis of gynandromorph production on an earlier observation that he had made with the sea-urchin eggs. He found that occasionally the egg-nucleus began to divide before the sperm-nucleus had fused with it. In consequence, the sperm-nucleus fertilized, as it were, only one-half of the egg; *i. e.*, it approached one of the two daughter nuclei, and later became incorporated with that one. In consequence, all the nuclei descending from this fusion had the diploid number of chromosomes, while the nuclei descending from the single daughter egg-nucleus had only the haploid number. In the sea-urchin it has not been found possible to raise plutei to maturity; hence the effect of this partial fertilization on sex could not be determined. Boveri's application of this evidence to gynandromorphs of the bee was purely theoretical, since at that time the genetic evidence, that has since become available, did not exist.

At about the same time Herbst carried out some experiments with sea-urchin eggs that enabled him to produce a large number of em-

bryos in which a process similar to that just described took place. The unfertilized eggs were stimulated to parthenogenetic development by placing them in sea-water containing a little valerianic acid. After a few minutes the eggs were returned to sea-water and sperm added. The sperm-nucleus did not penetrate in many cases until the egg nucleus had begun to divide and then, as in Boveri's case, it often united with one of the daughter nuclei. In neither of the cases is there any elimination of single chromosomes, but in a more general sense the earlier group of paternal chromosomes was dislocated in that it failed to reach its normal destination.

The extremely important experiments that Baltzer made with sea-urchin eggs resulted in demonstrable cases of elimination, but here of whole undivided chromosomes. For instance, when the eggs of *Strongylocentrotus* are fertilized with the sperm of *Sphærechinus*, it is found at the first division of the egg that while some of the chromosomes divide and the halves move to opposite poles, other chromosomes remain in place, or become scattered irregularly between the two poles of the spindle. They appear later as irregular granules and show signs of degeneration, and although remnants of them may persist for a while, they take no further part in the development. The maternal egg-nucleus contained in this case 18 chromosomes and likewise the paternal sperm-nucleus. Hence, after union and division, 36 chromosomes should go to each pole of the segmentation spindle if all divided. Baltzer found, however, only 21 chromosomes at each pole, which means that 15 chromosomes have failed to behave normally, and it is probable that these are derived from the paternal nucleus. Three chromosomes only of the latter, on this interpretation, take part in the division. In consequence, the nuclei of the embryo contain almost exclusively maternal chromosomes, and it is significant that the larvæ are largely or entirely maternal in character. It is true that we have no evidence to show at present that the larvæ of these sea-urchins differ in only one or more Mendelian factors. It would be very surprising if such were the case, yet the results show at least so great a preponderance of maternal characters that we must infer that the three surviving paternal chromosomes produce no marked difference.

The reciprocal cross gave a different result. When the eggs of *Sphærechinus* are fertilized by the sperm of *Strongylocentrotus*, division of all of the chromosomes takes place normally and 36 are found at each pole. The pluteus that develops shows peculiarities of both paternal and maternal types. The difference between the two crosses is probably due to the observed differences in the behavior of the chromosomes. In the first case, the lagging and subsequent degeneration of certain chromosomes may be spoken of as a sort of elimination, although the causes that bring it about must be supposed to be of a

different kind from those involved in *Drosophila* when a half of a single chromosome fails to reach its normal destination.

EARLIER HYPOTHESES TO EXPLAIN GYNANDROMORPHS.

Dalla Torre and Friese (1897) and Mehling (1915) have reviewed the earlier attempts to account for gynandromorphs. Dönhoff (1860) suggested that gynandromorph bees arose from eggs with two yolks, one of which was fertilized, the other not; one began to form a worker, the other a drone, both fusing into one individual later. A second interpretation based on Dzierzon's theory was also suggested, viz, that the egg contains the male potentiality, the sperm the female potentiality. In fertilized eggs the latter influence usually predominates. In the gynandromorph, one of these influences predominates in one region, the other in other regions. In 1861, Wittenhagen suggested that a queen that produces gynandromorphs has reached a higher stage of fertility which causes male parts to arise even after fertilization. Menzel (1862) made several guesses, such as that delayed fertilization of the egg leads to irregular distribution of the mass of the sperm material with consequent disturbance in the development. Later (1864) he suggested that abnormal organisation of the oviducts, leading to delay in passing of the egg, interferes with the sperm, so that the egg no longer has the possibility of producing a complete female, except in certain regions of the body.

Von Siebold (1864) thought that insufficient fertilization is responsible for the appearance of gynandromorphs. He assumed that a definite number of spermatozoa are necessary to produce a female. When from any cause an insufficient number of sperms is present, the egg can not develop a female, or a male, but an intermediate type.

According to Cockayne (1915, p. 117), Scopoli (1777) suggested that a gynandromorph of *Phalæna pini* might have arisen through the fusion of two pupæ lying in one cocoon. Dönhoff's suggestion (as above) of two yolks in one shell that fused is a somewhat similar view, and Wheeler in 1910 made a like suggestion, viz, that two eggs (fertilized?) fused at a very early stage, one a male-producing, the other a female-producing. Such a process will not apply, however, to most of the cases in *Drosophila*, because the evidence shows that the eggs are normally not of two kinds. The male alone produces two kinds of gametes. The sex-linked characters in hybrid gynandromorphs show very clearly that the results are not due to the fusion of two eggs, but to a different sort of process. In the bee also it appears that there is only one kind of egg, and that the female sex is determined by the fertilization of the egg; the male comes from the unfertilized egg.

On the other hand, there are several cases in *Drosophila* which can not be explained by simple chromosomal elimination, but which can be explained on the assumption that the egg had two nuclei. Here

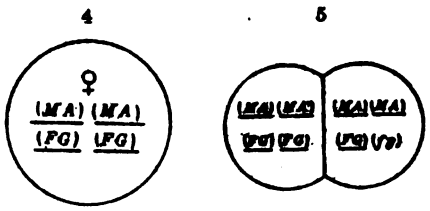
the appeal is made to a binucleated egg in order to account for the distribution of the sex-linked characters, but only indirectly for the sex differences in the gynandromorph. The different sexes in the two parts are due to fertilization of the two nuclei by male and female producing sperm respectively. The presence of two nuclei in these eggs is easily explained as due to the fusion of two oogonial cells or else by an oogonial nuclear division without cytoplasmic division. The conditions existing at the completion of the last oogonial division are particularly favorable for such a union, for at this stage from a collection of cells (presumably all alike) the most favorably situated turns into the egg and the others into nurse-cells very intimately connected with the egg-cell. This view, while similar to Wheeler's, puts a different emphasis on the facts, for here the presence in the eggs of two nuclei does not directly account for the different sex of the parts of the gynandromorph (for this difference is due to the two kinds of sperm that have entered), but explains the distribution of the sex-linked characters in the hybrid gynandromorphs. On the other hand, Wheeler's idea is that two eggs in themselves determined as male and female fuse bodily, *i. e.*, side by side, to give rise to male and female parts respectively. His view would be more nearly realized in the case of moths where the female is the heterozygous sex, and consequently a binucleated condition can be utilized directly to explain not only the difference of sex in the gynandromorph (one nucleus retaining a Z and the other a W chromosome), but also the autosomal mosaics, as in the cases described by Toyama.

Arnold Lang suggested another possibility in 1912, viz, that an egg that had developed parthenogenetically to the stage when the first two nuclei were formed might be fertilized by a female and a male producing sperm, each sperm uniting with one or the other of the two egg-nuclei. As a result one half should be male, the other half female. The hypothesis will not apply, however, to the bee—the forms whose parthenogenetic process of development would seem to best fit such a view—because only one kind of sperm is supposed to be produced. Double nuclei should produce female parts. The explanation will also obviously not apply to such cases in *Drosophila* as those in which the male half shows maternal recessive factors.

De Meijere (1910–11) has offered certain suggestions concerning the origin of gynandromorphs. He starts from the old idea that each individual, male or female, contains within itself the characters of the opposite sex. He thinks that this holds for the gametes as well as for the somatic cells. Darwin held a similar view and thought that this was true not only for the primary sex-cells (sperm and eggs) but for the secondary sexual characters as well. To-day, however, it is clear that such a statement, at least in regard to the established cases of sex determination by means of sex factors, calls for a more definite

pronouncement as to the sense in which the phrase is employed; otherwise it is little more than a play on words. For instance, when one X chromosome is present the individual is a male, which means that one X plus all the rest of the cell makes a male, and when two X's are present, these two plus all the rest of the cell make a female. In what sense can such a statement be twisted to mean that each such combination contains in a latent condition the opposite condition? Compare the facts with a similar chemical situation and the absurdity of the inclusion hypothesis is evident. Maltose has the formula $C_{12}H_{22}O_{11}$ and glucose the formula $C_6H_{12}O_6$. One is twice the other minus one H_2O. To state that maltose contains glucose latent or that glucose contains maltose latent is obviously absurd, yet this does not differ much from the view that each sex contains the opposite one in latent form.

De Meijere thinks that gynandromorphs can be explained in "that the activation of the opposite sex (opposite to the one already under way) has started in, relatively later, after all the parts have taken on their definite positions; many of the parts have gone too far in the first direction, *i. e.*, they are too old, but those that have not may be turned aside and produce the opposite results."[1] This view is offered to account for mosaics of sex character. The bilateral gynandromorph, he supposes, owes its origin to the above changes having taken place very early, even at the first division. De Meijere thinks apparently of effects being produced by external factors of some unknown kind rather than internal ones connected with a sex mechanism. His idea is too vague to be of use and too remote from present-day knowledge about sex determination to call for extended criticism.



TEXT-FIGURES 4 AND 5.

Arnold Lang, accepting the same general conception of sex and expressing what he believed to be the real relations by means of the formulæ that Goldschmidt had advocated, offered another possible interpretation of gynandromorphs that is superficially exactly like the theory of chromosomal elimination which the results in *Drosophila* show to hold for this insect. In fact, Lang's view, if divested of the unnecessary encumbrance of De Meijere's conception and of Goldschmidt's formulæ, is then identical with the theory of chromosomal elimination. For example, Lang represents the fertilized egg (one that will give rise to a female) by the scheme shown in text-figure 4. The primary sex characters for the male are M carried by a pair of

[1] See Goldschmidt's view in respect to the rate of development of male and female organs in the intersexes of the gipsy moth.

chromosomes that also carries the factors for the secondary sexual character A. The primary sex character of the female is represented by F, carried by a second pair of chromosomes, and the secondary sexual character by G, both as before, carried in the same chromosome. In other words, the two pairs of sex chromosomes are (FG) (FG) and (MA) (MA) for the female, and, for the male, (FG) (fg) and (MA) (MA).

Lang suggests that a loss by *mutation* takes place in females (as above) in the sense that one FG disappears and may now be represented by (fg). The resulting division is shown in text-figure 5. The mutation causes the sex-balance in the cell on the right side to turn into a male, while that of the left remains a female. Lang appears to mean that the "mutation by loss" is the loss of a daughter chromosome.

If we ignore the special interpretation of sex employed by Lang and borne out by his formulæ, his view has several points in common with the hypothesis of chromosomal elimination. It should be noted, however, that there are also differences in the application of Lang's and the present interpretation, when the question of the sex-linked factors is involved, because the two X chromosomes represented by Lang by FG, FG carry many other genes, besides those for sex, even some for secondary sexual characters. Which of these comes to expression in the hybrid gynandromorph depend on which FG is eliminated and not on the resulting change in balance (epigenetic effects) between the FG's and the MA's. Furthermore, Lang's scheme involves the relation between two pairs of chromosomes (four in all) while in the actual case of *Drosophila* only one pair is needed to account for all the facts.

Cockayne, in 1915, announced independently the same view of elimination that Morgan had published the year before. He had found several halved gynandromorphs, all of which showed the specific characters of both parents on both sides. Both parental nuclei must therefore have contributed to both sides. He points out that since the division into male and female parts sometimes coincides with other characters the latter must be carried by the sex chromosomes.

Doncaster, in 1914, described binucleated eggs in *Abraxas*, each nucleus giving off its two polar bodies and each being independently fertilized. He suggests that gynandromorphs might arise from such eggs, but did not obtain any in the particular lines that showed such binucleated eggs. The two gynandromorphs in *Abraxas* that Doncaster described later (1917), and which are considered here on page 85, he did not attempt to explain by this condition.

The gynandromorphs of *Drosophila* have been from the time of their first appearance in our cultures, about 8 years ago, a subject of general interest and discussion, especially by Muller, Sturtevant, Bridges, and Morgan. Their relation to the gynandromorphs in bees and to the theories of the origin of the latter has been constantly

under discussion. The critical evidence that shows that they were not due to separation of whole maternal and paternal nuclei was first obtained and published by Morgan (in 1914). Prior to that time Bridges (1913) had published an account of two hybrid gynandromorphs, and had suggested that they were due to somatic non-disjunction. By this term it was meant at the time that at an early embryonic division of a female the two daughter halves of one of the X chromosomes did not disjoin from each other to pass, as normally, into sister cells, but were included in the same cell, the other cell not receiving its half. The non-disjoining X was assumed to divide normally and the result was an X cell developing into male parts and an XXX cell developing into female parts. This hypothesis served to explain all the facts known at that time. Soon, however, it was established (Bridges, 1916) that XXX individuals are unable to survive, and this brought into question the conclusion that the female parts of gynandromorphs were XXX. This difficulty was later avoided by the assumption of "elimination" (earlier called "mitotic dislocation," Morgan, 1914). As already stated, this meant that one of the daughter X's was caught by the mid-plate and prevented from taking its place in either nucleus.

There is another class of gynandromorphs (including here four cases) in which another procedure may account for the results. Primary equational non-disjunction occurred, as evidenced by the presence in each of the four gynandromorphs of two X chromosomes from the mother, one of these being a non-cross-over and the other a cross-over X, as is usual for XX eggs produced in this fashion. This XX egg was then fertilized by an X sperm, giving an XXX individual. This XXX zygote is prevented from dying and at the same time converted into a gynandromorph by the occurrence of somatic reduction at the first or a very early embryonic division. In each of the four cases the male parts of the gynandromorph were derived from one of the two maternal X's, which suggests that the essential feature of this somatic reduction is the active separation of the two X's that came from the mother and the passive inclusion of the X from the father with one or the other of them. There have been other cases which may support this view, cases in which XX eggs equationally produced have been fertilized by Y sperm, and then the two X's have likewise reduced, with the result that each cell gets one X, and the entire individual is converted into a male which is a mosaic of different parts clearly marked by the character corresponding to the two different X's. The difficulty with this view is that it assumes that reduction can take place between two X's at a cell division without the X's themselves splitting, although all of the other chromosomes do so at this time—a situation for which no support is given by cytology. It is to be noted in this connection that all cases that appear

to belong to this category are also explained by the assumption that the egg started with two nuclei, and in the description of cases both of these views are given as alternatives.

THE ORIGIN OF THE GERM-CELLS IN FLIES.

In several species of flies (*Miastor*, *Chironomus*, *Calliphora*) it is known that the germ-cells of the ovary or testis arise from a single cell at an early stage in the cleavage. In *Miastor*, for instance, when the four first-formed nuclei divide, one of the eight daughter nuclei moves to one pole of the egg, where it becomes surrounded by the peculiar protoplasm of this pole and subsequently pinches off from the surface of the egg. From this single cell by later division arise all of the germ-cells. A similar process has been described for other species of flies. If this holds also for *Drosophila* it follows that all of the germ-cells must be either eggs or sperm, regardless of whether the somatic parts are male or female. On the other hand, if the germ-cells in *Drosophila* and in the bee are formed as in some of the other insects, *i. e.*, in the beetle *Calligrapha* described by Hegner, where 16 cells simultaneously reach the polar field, it would be possible for some of the cells to have descended from one of the first two segmentation nuclei and some from the other. In such a case, if the first-division figure underwent elimination, both ovaries and testes might appear in the same individual. In butterflies and moths, where many gynandromorphs have been dissected, several cases in which both testes and ovaries occur are known. This is also the case in bees. A difference in the time of isolation of the germ-cells in these groups and in *Drosophila* may account for the difference in the results.

COURTSHIP OF GYNANDROMORPHS.

Sturtevant's paper on sex recognition and sexual selection in *Drosophila* gives a full account of the rather elaborate courtship of this fly, in which the behavior of the two sexes is quite different. The reactions of an animal, male on one side female on the other, or of one that had a female head and a male abdomen, might be expected to furnish interesting conclusions as to the relative importance of the sense-organs *versus* the reproductive organs in the behavior during courtship.

Sturtevant tested 6 gynandromorphs. One was male throughout, except the genitalia, which were female. It behaved as a male. Sections of the abdomen showed one abnormal egg present. Another had 2 sex-combs, right and left, and the right wing was shorter than the left. The abdomen was female. She produced at least 1 egg. Sections of the abdomen showed 2 large eggs and a degenerate ovary present. She courted and was courted, thus giving both reactions. A third was

male, except the genitalia, which were female. Sections showed an abnormal testis near posterior end. It courted and was courted.

Sturtevant records observations on three other gynandromorphs tested for sexual behavior:

"None showed any certain indications of male behavior, but all were vigorously courted by males. Of these three gynandromorphs the external characters were as follows: (A) All female, except one side of the head, which was male; (B) female on one side of the whole body, male on the other side; (C) female, except the genitalia, which were male."

Duncan describes the behavior of a bilateral gynandromorph. Its mating instincts were found to be indifferent. It was courted by males but would not court females. The gonads were both testes with ripe sperm. In a second gynandromorph, the eyes were female, but the forelegs had sex-combs; one wing was long (female); the abdomen was male type, but the genitalia were half male, half female. Two ovaries were present. The fly was courted "assiduously" by males but would not mate. A third gynandromorph was without sex-combs on the forelegs, the wings were the same length, but the abdomen was male on one side, female on the other, as were the external genitalia also. Mature sperm were present in both testes. This fly was anteriorly female and posteriorly half male and half female. A normal male courted this gynandromorph when in front, but did not copulate with it.

The gynandromorph drawn in text-figure 34 was tested by one of us (Morgan, T. H., Amer. Nat., 1915, p. 246). One side of the head and thorax is male, the other side female. The abdomen is pigmented above as in a male and there is a penis below. When put with mature unmated females it did not court them, although it was quite active.

Attempts to breed from gynandromorphs have been often made. It was not to be expected that those in which the genitalia were mixed would successfully copulate. Those with female abdomen have more often given offspring. Since, as explained elsewhere, the gynandromorphs with male abdomen would not be expected to be fertile (because the XO combination has been shown to be sterile), the frequent failure to obtain offspring from such males is in accordance with expectation. On the other hand, an occasional fertile male gynandromorph occurs. In these cases the combination was known or suspected of being XXY, the presence of the Y chromosome making the male (XY) fertile.

PHOTOTROPISM IN MOSAICS WITH ONE WHITE AND ONE RED EYE.

On several occasions it has been observed that when a mosaic had one red and one white eye it circled to the red side. This behavior is expected from observations by McEwen on the light reaction of flies

from white-eyed stock. He showed that these flies respond much less actively to light than do red-eyed flies. In these red-white mosaics the red eye, giving a stronger positively phototropic reaction, turns the fly toward that side. Of course, if the fly turns toward a single source of illumination, such as a window or artificial light, the red eye will soon pass into its own shadow as the fly turns, and the condition on the two sides may become balanced, unless the general illumination from the wall of the room, for instance, is still stronger than the influence of the window's light on the white eye. In order to avoid this complication the fly should be kept on a vertical surface held at right angles to the light, when its circus movements are not interfered with by the opacity of its own body.

Since the male side of the body, including the legs, is generally smaller than the female side, and since the male side is the one that has the white eye, there is a chance that the movements toward the red side are against the stronger action of that side. This complication was, however, not realized in all the cases in which circling occurred, but since in several of them the legs on the right and left sides were the same it is practically certain that the results are largely, if not entirely, due to the difference in stimulus from the two eyes.

SEX-LIMITED MOSAICS.

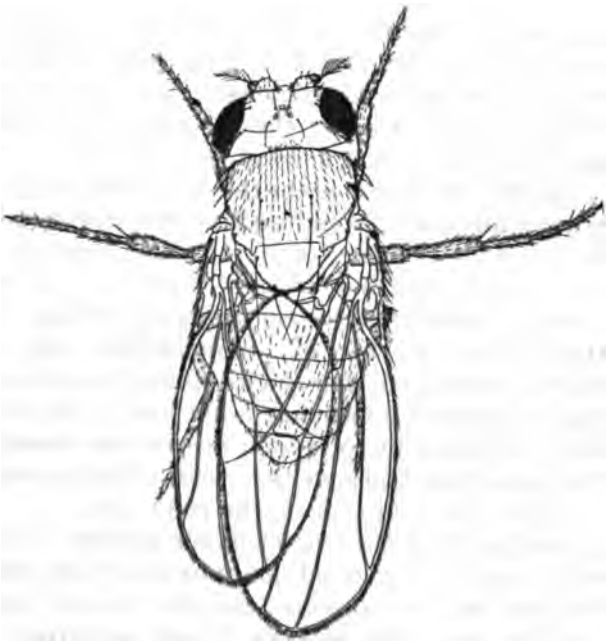
By a sex-limited character (in contradistinction to a sex-linked) we mean a character that is peculiar to one or the other sex, but is not necessarily transmitted by means of a gene in the sex chromosome. Such a character is shown by a stock called white tip, in which the pigment bands are absent from the last segments of the abdomen in the female but not in the male. In this stock a gynandromorph arose (text-fig. 6), male on the left side and female on the right. On the male side the black tip to the abdomen is present, although here, as in the stock itself, it is not as black as in the wild type. On the female side the abdomen has a white end.

In this case elimination of a sex chromosome produced the gynandromorphous condition, and since in this stock the female parts are different from the male, owing to a factor presumably not in the sex chromosome, the right side of the gynandromorph also shows this peculiarity, owing to its femaleness.

A similar case appeared (No. 2864, Jan. 1915) in a cross between a faint-band female and a star faint-band male. Faint-band is a sex-linked character which appears only in the female. All of the flies of the above cross were pure faint-bands; but while the females were characterized by abdominal bands in which both chitinization and pigmentation were weak and by short, slender, and irregular bristles throughout, the males could not be distinguished from wild males in appearance. The gynandromorph was completely bilateral, the

right side being male, with sex-comb, smaller eye, wing, etc., and the right side of the abdomen with male coloration. The genitalia were half-and-half also. The interesting feature was that throughout the female left side the bristles were weak and irregular and the bands "faint," while the male right side was entirely wild-type in appearance.

Another striking case appeared (on March 23, 1916) among the offspring of a pair, the female of which was heterozygous for the sex-limited character ("side-abnormal") and the father was pure for it. The character "side-abnormal" is sex-linked in inheritance and sex-limited in appearance, being seen only in females. In this mutant the bands of the abdomen of the female are "abnormal" at the sides, *i. e.*, while the mid-dorsal part of the band is normal the ends of the band where they come around the side are cut away irregularly to ragged points and the color is etched with white splotches in the dark. The ventral plates are much smaller and are irregularly rounded. In the male all parts are as in the wild flies. This gynandromorph (3806) showed a normal male right half of the abdomen and a female left half, with all the characteristics of the side-abnormal character. The ventral plates were full and normal in the male parts and small and irregular in the female parts. Other evidences of maleness were present—a sex-comb on the right foreleg and a smaller right wing.



TEXT-FIGURE 6.

Elsewhere in the text we have described several other cases involving characters both sex-linked and sex-limited. Thus in gynandromorph 7530, page 46, the male eye on the right showed marked development of the character facet, as in the normal facet male, while the female left eye, also facet, could hardly be told from wild-type, as is usual in facet females. All gynandromorphs involving eosin eye-color show the light type of eosin in the male eyes and the dark type in the female eyes.

SOMATIC MOSAICS.

Somatic mosaics can be accounted for by autosomal elimination in the same way that gynandromorphs are accounted for by X-chromosomal elimination. Somatic mosaics might also be expected to arise from binucleated eggs and to be as often found as are gynandromorphs with the same origin. As a matter of fact, we have found only one certain case, which is less than expected on the latter view. The case is as follows:

The grandmother was spineless (third-chromosome recessive) and the grandfather was spread (another third-chromosome recessive). The daughters and sons were wild-type. A pair of these gave a 2:1:1:0 ratio, as expected, because of no crossing over in the male.

One of the granddaughters (No. 561, Oct. 3, 1914, text-fig. 7) was a mosaic of spineless and not-spineless. The left side of the thorax and abdomen and the left wing and the middle and last left leg were spineless. The rest of the female (including all of the head and left foreleg) had long bristles and hairs of the wild type.

TEXT-FIGURE 7.

Simple elimination of the third chromosome from the spread parent would explain this case were it not that the existence of an individual lacking an autosome is doubtful, because none have as yet appeared through autosomal non-disjunction. On the alternative view of a binucleated egg, one nucleus contained the spineless third chromosome, the other a spread-bearing chromosome; both nuclei were fertilized by X sperm bearing the spineless X chromosome, and gave the female spineless on the left side and wild-type on the right side.

The fact that the overwhelming number of hybrid mosaics are gynandromorphs, involving therefore the sex chromosome, can not be explained as due to failure to discover autosomal mosaics if they occurred. In most of our cases these would be just as striking as in the cases where the sex chromosomes are involved. Evidently some peculiarity in the separation of the halves of the sex chromosomes makes the elimination of one of the daughter halves more probable than in the case of other chromosomes. Such a supposition is, of course, in harmony with the peculiar behavior of the sex chromosome at the reduction division of the male, at least when it lags on the spindle. On the other hand, when it does divide, as in the female, no such peculiarity is recorded, and it is this reduction, rather than the former one, that we need for comparison.

SOMATIC MUTATION.

That mutation may take place in somatic cells comparable to the mutation process in the germ-tract can not be doubted. The bud-sports long familiar to botanists probably furnish in some instances examples of this sort; but the best authenticated cases are the modern ones that have been analyzed by recognized genetic methods. Few examples are known to zoologists; the monsters, freaks, and duplications that are frequently found are generally due to environmental effects on the embryo.

If somatic mutation occurs in only one chromosome of a pair, as seems to be the case with germinal mutations, the immediate result will not be seen except when the mutation is dominant. In the case of mutation in the germ-tract, a recessive gene in one chromosome of a pair may likewise not have opportunity at first to express itself, but if it is carried to one of the offspring it will there become multiplied and get into daughters and sons (or in hermaphroditic species into pollen and ovules). Chance union of the gametes that contain the mutated chromosomes will later bring even the recessive genes to expression. It is more probable, therefore, that recessive mutations will appear in the sexually reproducing species more readily than in those with vegetative reproduction, except where the latter are already heterozygous. The same comparison may be made between parthenogenetic species and sexual ones. In the former, a recessive mutation appearing in one chromosome of a pair will have no opportunity to show effects, and the line may be lost by chance alone. Preservation will be favored only if the heterozygous state has an advantage over the original form. Sexual reproduction, therefore, has the advantage that every recessive mutation will have a far better chance of showing itself as a character modification and, if beneficial, of being preserved by natural selection. In fact, if it could be shown that a preponderant number of recessive mutations have furnished the material for evolution, it might possibly appear that we had some hint as to how the process has come to be such an almost universal method of propagation. On the other hand, dominant mutations might flourish, as well by the one as by the other method.

The best authenticated case of somatic mutation in plants is that described by Emerson, who has brought forward convincing evidence that in corn a gene for certain types of variegation (striped seeds) mutates not infrequently to a gene for uniform-colored grain. The gene for medium variegated "mutates much more frequently than that for very light variegation." By crossing plants from the mutated grains to pure recessive types Emerson has shown that when the mutation occurs it involves only one member (at a time) of the pair of allelomorphs in question. In these cases the mutation takes place in cell lines (subepidermis) that may ultimately contribute both to

the germ-tract and to the soma. Through the former, inheritance becomes possible, through the latter the effects of the mutation become visible only on the plant in which the mutation took place. There are other mutative changes in corn that Emerson describes in which the effect is only in the epidermal cells; hence, while it becomes visible in the plant in which it has taken place, it is not inherited, since the germ-tract does not come from this part of the plant.

In the course of our work on *Drosophila* a few flies have appeared with characters which seem to have arisen by somatic mutation. If, as there is reason to suppose, the mutation changes that gave rise to them appeared in only one chromosome, the change must either have been dominant or, if recessive, in the single X chromosome of the male. Since visible mutations in the sex chromosome have been shown to be at least four times as frequent as dominants in all of the chromosomes together, the chance that these sporting characters are dominants is smaller than that they are recessive and in the sex chromosome. In support of the latter is the fact that nine out of ten of the sporting characters look like known sex-linked genetic characters, and more important still is the fact that all the cases so far found are males.

(1) One of these somatic sports is shown in plate 1, figure 4. The right side of the body is pale, almost white. The history of this fly is as follows:[1]

One of the X chromosomes of the mother contained the genes for lethal 7 and for forked, the other X the genes for yellow and for white. The X chromosome of the father carried the genes for yellow and for white. The fly was a yellow white forked male throughout, but the right side of the thorax, the right wing, and the right side of abdomen were pale, almost white, as shown in the drawing. Testes were present, with sperm. The pale light side is clearly due to somatic mutation, since no such pale body-color was present in the cross or was known elsewhere. Whether the mutation occurred in the X (if recessive) or in an autosome (if dominant) is undeterminable, since the fly was not bred.

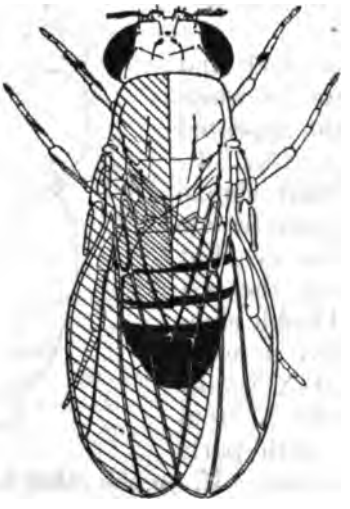
(2) In another case (II 108, Oct. 21, 1913), the left side of the body, at least for a middle section, is brown in color, looking like the double recessive yellow black (text-fig. 8). The fly had the following history:

Some F_2 blacks from the cross of black by jaunty (both second-chromosome) were inbred in an attempt to secure the double recessive black jaunty. One of the F_3 black males had the left side of its thorax and abdomen, left wing, and left legs colored like the double recessive yellow black. It was at once assumed that mutation to yellow had occurred in the early embryo in the cells which gave rise to the left side. A test was made to see whether the germ-cells carried the mutant gene. The mosaic male was outcrossed to a black female and gave only black offspring (M 69, black ♀ 27, black ♂ 23). Three pairs and a mass-culture of these F_1 flies were inbred and gave a total

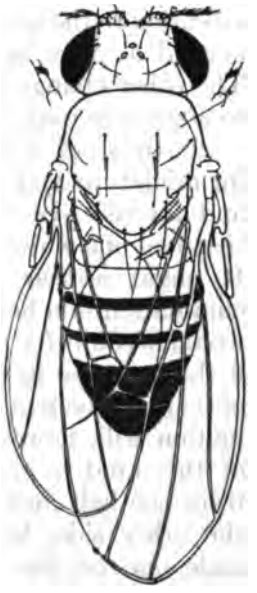
[1] No. 2493; November 20, 1915.

of 152 black ♀ and 147 black ♂, with no yellow-black offspring. Evidently, then, the testes came from a cell which had not mutated. While the "brown" color of the mosaic was like that produced by yellow acting with black, it is possible that the mutant gene was not the yellow already known, but a new yellow.

(3) Among the grandchildren of the last somatic sport a fly was found with a wing of an unusual type (text-fig. 9). This wing was about half the usual length and had almost exactly the form of miniature, but there was none of the dark color normally present in miniature wings. This wing seems to have been a new mutant type, the mutation having occurred in the early embryonic cells of the fly. There have been quite a number of such occurrences, some, as in the present case, giving striking differences.



TEXT-FIGURE 8.



TEXT-FIGURE 9.

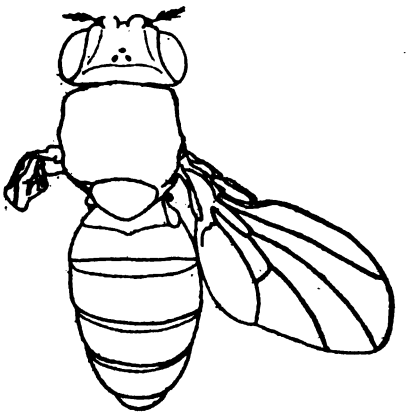
(4) A fly appeared in vestigial stock (August 13, 1912) with one normal wing (text-fig. 10). It was described as a case of somatic atavism. An alternative view is also possible, viz, that a somatic mutation occurred elsewhere, *i. e.*, in another chromosome or in another region of the second chromosome, of such a sort that it neutralized the effect of both genes for vestigial. In the cells containing this mutant gene the conditions for normal wings are again restored.

(5) and (6) Two further cases of mutation in the male were found by Sturtevant (not published); both were males throughout; one had forked bristles on one side of the body, although there were no forked flies in the immediate ancestry. The other had a dark body-color on part of the thorax, there being no sex-linked dark body-color in the pedigree. Neither fly was tested.

(7) In a stock pure for red eyes, miniature wings, and yellow body-color a fly appeared with all the characters of its race except that one eye was white with a fleck of red at its posterior edge (text-fig. 11).

Since there was no white in the stock, the white eye must have come by mutation and possibly by mutation to a sex-linked white-eyed gene.

(8) In a mating in which both parents were pure bar-eyed flies a male appeared (1917) (text-fig. 12) in which both eyes were round and in addition one eye was three-quarters white, and the other had a fleck of white in it. A germinal mutation in the mother of bar to round eye must have taken place, as shown by the fact that when the fly was bred it produced only normal-eyed offspring. Since this male was normal, it must have come from the union of a Y-bearing sperm and an X egg. Since the bar gene is carried by the X chromosome, it follows here that mutation must have occurred in one sex chromosome of the mother. It is significant in this connection to call attention to the fact that bar-eye not infrequently mutates (reverts) to normal, as May has clearly proven. The other change to white was due to a somatic mutation.



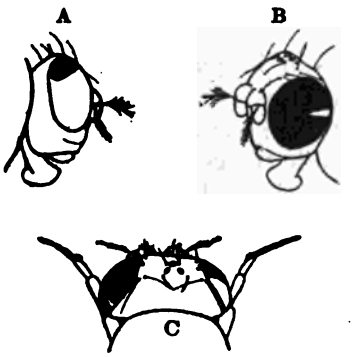
TEXT-FIGURE 10.

(9) In stock pure for black and for miniature and impure for white and for red eyes a male appeared that had one white eye (text-fig. 13). It might appear here that simple elimination in a heterozygous female would account for the white eye, but if the fly arose in this way the rest of it should be female. Double elimination will, however, give a result of this kind, *i. e.*, a red X is lost from one half and a white X from the other side, leaving both parts male, one red, the other white. If, on the other hand, the fly started as a red-eyed male and dislocation occurred, so that most of the fly had an X, the other part a Y chromosome, the expectation, based on the evidence from nondisjunction, would be that the male part would die. However, it might be claimed that the evidence applies to the fly as a whole and not to the survival of a small part of the body, which might very well be capable of living. But we should expect the absence of X to carry other consequences in its train besides loss of eye-color, so that this explanation seems improbable. A third explanation is that of somatic mutation. It is not possible to decide between the assumption of double elimination and that of somatic mutation.

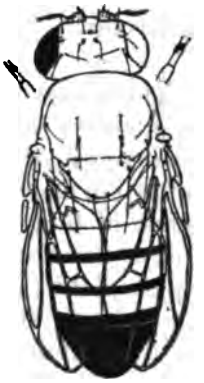
(10) A somewhat similar case is shown in the male figured in plate 1, figure 5. Its ancestry is not now a matter of record, but probably it arose in red-eyed bifid stock that we had at the time. If so, double elimination is excluded and the fly must have arisen by mutation in the sex chromosome.

It is a matter of great interest to find that all the ten cases of *somatic* mutation that we have recorded in *Drosophila* have been males. The significance of this was not appreciated until the material had been sorted out for other purposes. It probably means that a *recessive* somatic mutation takes place in the sex chromosome and shows at once in a male in those parts of the body whose cells contain the mutant gene because the male has only one sex chromosome. Should a recessive mutation occur in the X chromosome of a female its effect would not appear in the soma because the normal allelomorph would conceal it. It is interesting to apply this point of view to certain results in Lepidoptera in which mosaics or gynandromorphs have been recorded that carry in parts of the body characteristics that are known to occur, although rarely, in varieties of sports of the species.

TEXT-FIGURE 11.



TEXT-FIGURE 12.



TEXT-FIGURE 13.

Among these a number have been described with one half of the body of one species and the other half of a varietal type of the same species. In some cases the variety is so rare that there might seem to be no question of a hybrid cross involved, since this in itself would be rare, and that both this and a later mosaic condition result is beyond reasonable probability. An alternative view would be that of somatic mutation. If such were the explanation we should expect the individual to be female and the mutation to have occurred in the single Z chromosome.

In the cases brought together by Cockayne, in which the same individual is partly one species, partly a variety (1915, pp. 87–90), there are about 10 such cases recorded as females, 2 as males; in 12 cases no sex is stated by Cockayne. If further examination of the original sources shows as high a percentage of females as in the recorded cases, the evidence is in favor of the interpretation suggested above. The males call for another interpretation, and each such case will need special examination.

These cases are not to be confused with mutation in the germ-tract, where, in a sense, the reverse situation is realized, for while in *Drosophila* the mutation of a sex-linked character in one female chromosome appears immediately in one (or more) of her sons, the mutation itself occurred first in the female. Conversely, in moths, if a germ-tract mutation took place in the male it would show immediately in one or more daughters. The well-known case of *Abraxas grossulariata* may be taken to show why mutation taking place in a male is expected to show first in the female and not in the male offspring. The genetic evidence for *Abraxas* indicates that the female has one sex chromosome, the male two. The aberrant form *lacticolor* is found occasionally in nature and is always female. A mutation to *lacticolor* in a Z chromosome of the male would give rise to a daughter if this sperm fertilized a not-Z egg that would at once show the sex-linked character lacticolor.

MOSAICS IN PLANTS.

The cause of variegation in plants is too involved and obscure[1] to attempt to discuss in this connection. On the other hand, the occurrence of bud-sports is generally recognized as due to somatic mutation which may include the germ-tract also. The frequent occurrence of bud variation in the cultivated forms of the foliage plant *Coleus* has recently been studied by Stout, who has obtained from a single plant (and its clones) a number of types differing both in color and form of the leaves. The cultivated varieties have arisen through hybridization. Three interpretations suggest themselves as possible. Elimination of the chromosomes of the hybrid might account for the results, but no information as to the chromosomes in the different types is available. If any of the colors are due to cytoplasmic plastids, their irregular distribution might also be responsible for the result. Thirdly, the change might be due to a mutation. If the types studied are complex hybrids with one or more heterogeneous pairs of chromosomes, a change in one gene of one chromosome might bring about directly a visible change in the color. Until more critical Mendelian work is done it is not possible to reach any plausible or even probable conclusion. It might be possible to analyze the results more closely if we knew what kinds of offspring arise from the original plant and its varieties. Owing to the complex nature of the plants this procedure offers difficulties. A few facts are given by Stout. He states that "plants grown from seed give wide variations . . . . Many of the types that had appeared as bud variations appeared also in the seed progenies."

Winkler produced mosaics by grafting tomato and nightshade, which are now supposed to be due to a combination of the tissues of the

[1] Except in the case of *Pelargonium* and of *Mirabilis*, where Baur and Correns have shown that the mosaics are caused, in some instances at least, by plastid assortments.

two plants—the epidermis of one species and a core of the other species. The mosaic shown in *Cytisus adami*, a hybrid resulting from grafting *Cytisus purpureus* and *Laburnum vulgare*, seems also to be due to a similar sort of combination. In animals mosaics have been produced in hydra by King by grafting pieces of a deep-green race on a light one, and by Whitney by destroying the green pigment of one individual and grafting pieces of it onto a normal green hydra. In tadpoles combinations of different species caused by grafting have been made by Born, Harrison, Morgan, and others. A result strictly comparable to the periclinal chimæras of plants has been reached by grafting a piece of the tail of one species on to the amputated stump of another species. As the new tail grows the skin of the stock is carried out over the core derived from the graft, and as a result an organ is formed with an outer layer of one and a core of another species.

The mosaic seeds of corn that are striped with red and white have been shown by Emerson to arise through a mutation in the gene for striping. The "half-and-half" mosaic grains that have been recorded by Correns (1899), Weber (1900), East and Hayes (1911), Emerson (1915), and Collins (1919) have been variously accounted for—recalling the different interpretations that have been advocated for gynandromorphs in animals. Emerson (1915) reviews these theories and advances the explanation of somatic mutation. It seems not improbable that elimination will account for those mosaics in which the triploid endosperm nucleus is involved.

CLASSIFICATION AND DESCRIPTION OF GYNANDROMORPHS OF DROSOPHILA.

The main group includes the gynandromorphs that are adequately explained by chromosomal elimination. It is subdivided according to the type of gynandromorph into: (1) those approximately bilateral, (2) those mainly female, (3) those mainly male, (4) those in which the type is largely "fore and aft," and (5) those in which the mother was known to have been an XXYfemale, but in which simple elimination is sufficient to account for the results. Another group (6) includes those in which the distribution of parts is irregular. These types are only approximations and by no means mutually exclusive; it is often somewhat difficult to decide to which type a specimen belongs.

The highly interesting group of special cases (7) is undivided, though it calls for three or four different genetic explanations, based, however, on special modes of distribution of the sex chromosomes. In the Appendix are included those cases in which our records are incomplete as to parentage or in which the specimen has been lost, so that the description is sketchy. This group contains many of the very early gynandromorphs. To this subdivision is added a brief review of previously published gynandromorphs in *Drosophila*.

Within each subdivision the arrangement of the cases is according to the order of discovery, that is, by date, except that the colored figures are taken out of order and described first in each group.

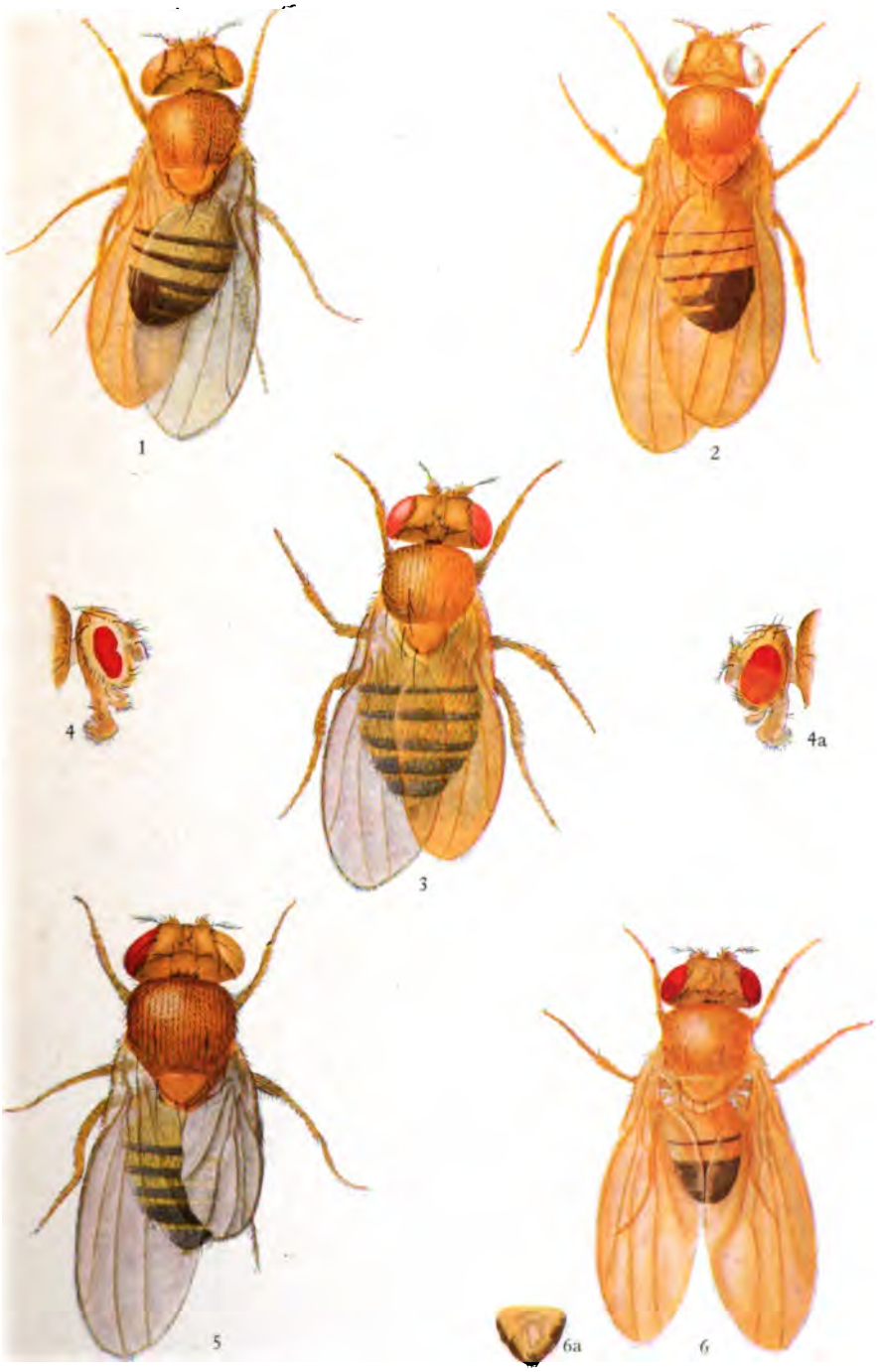
Each case is known by a number, which is usually that of the culture bottle in which the gynandromorph was found, but in some cases letters or small numbers are used, which, however, correspond to the bottle in which the specimen is preserved or the order in which the descriptions were first arranged. The date, the finder, and the type of illustrations are also indicated on the number line.

The information on each case is then given in the order, *Parentage*, *Description*, and *Explanation*. In many of the cases the explanation is followed by a diagram showing *at the left* the two X chromosomes of the zygote, which at the same time represent the female parts of the gynandromorph, and *at the right* the single X that is left after elimination, which gives the constitution of the male parts. In case somatic reduction was involved the leftmost set of chromosomes represents the initial condition of the zygote, and the other two sets to the right the resulting two conditions, whether male or female.

A knowledge of the order and the relative spacing of the genes along the chromosome is indispensable, and we have therefore made a list of the sex-linked mutants mentioned, with their symbols and the approximate locus of each:

Mutant.	Symbol.	Locus.	Mutant.	Symbol.	Locus.
Sable duplication...	S	0.0	Club.....	c_l	16.7
Lethal 6.....	l_6	−0.004	Cut.....	c_t	20.0
Yellow.....	y	0.0	Tan.....	t	27.5
Lethal 7.....	l_7	0.3	Vermilion.....	v	33.0
White.....	w	1.1	Miniature.....	m	36.1
Eosin.....	w^e		Lethal 9.....	l_9	38.0
Blood.....	w^b		Sable.....	s	43.0
Cherry.....	w^c		Garnet.....	g	44.4
Notch.....	N	2.6	Rugose.....	r_g	45±
Facet.....	f_a		Lethal 4.....	l_4	49.0
Bifid.....	b_i	6.3	Rudimentary.....	r	55.1
Ruby.....	r_b	7.0	Forked.....	f	56.5
Claret.....	$r_b{}^c$		Bar.....	B	57.0
Crimson.....	$r_b{}^{cr}$		Fused.....	f_u	59.5
Lethal 2.....	l_2	12.5	Cleft.....	c_f	65±

The figures in the plates are camera-lucida drawings of etherized living flies, but in the following descriptions of the gynandromorphs diagrams only are given (except in rare instances). These diagrams were made from the flies themselves, which are preserved in alcohol. All drawings and diagrams were made by Miss Edith M. Wallace, to whose skill and accuracy they bear witness.



E. M. Wallace Pinx

GYNANDROMORPHS OF DROSOPHILA

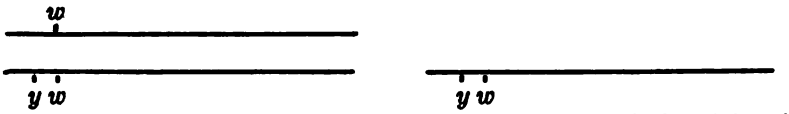
APPROXIMATELY BILATERAL GYNANDROMORPHS.

No. G_1ad_3. Feb. 1914. E. M. Wallace. Plate 2, Figure 1 (colored drawing).

Parentage.—The mother was a white-eosin compound female from the cross of a yellow white female to an eosin male. The father was a yellow white male.

Description.—The entire head, the right half of the thorax and of the abdomen, the right wing, and all of the right legs were gray and female. Both eyes were white-eosin compound and therefore female, which was in agreement with the gray color and black bristles of the whole head. The genitalia were apparently entirely female. The left side of the thorax and of the abdomen, the left wing, and all of the left legs were yellow in body-color, smaller, and male. There was a sex-comb on the left side only. The gynandromorph failed to produce offspring when tested.

Explanation.—An egg with the eosin-bearing X was fertilized by the X sperm bearing the genes for yellow and white. The zygote was therefore female, and the female parts of the gynandromorph have this constitution. At the first segmentation, division elimination of a maternally-derived eosin-bearing X occurred, giving rise to a cell with only a yellow white X. The parts descended from this cell were male and showed the yellow body-color corresponding to the yellow white chromosome. Since neither of the eyes was male, the white eye-color had no chance to show on the left side.

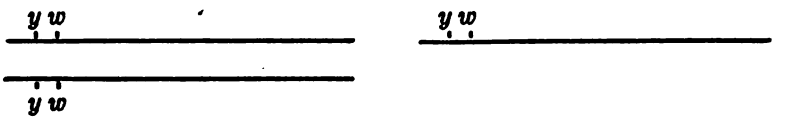


No. G_2C_{19a}. Feb. 1914. E. M. Wallace. Plate 2, Figure 2 (colored drawing).

Parentage.—A mass culture of the white-eosin compound females (eosin in one X and yellow white in the other, from gynandromorph G_2C_{19} above) outcrossed to yellow white males produced a further gynandromorph (G_2C_{19a}).

Description.—The gynandromorph, while yellow and white throughout, was a strict bilateral gynandromorph, including genitalia and antennæ. The right side was male throughout, as evidenced by sex-comb, smaller size of bristles and of all parts such as eye, thorax, wing, legs, and abdomen, and by the male coloration of right side of the abdomen. The gynandromorph was tested but gave no offspring. Sections showed slightly developed ovaries on both sides.

Explanation.—An egg containing the X with the genes for yellow and white was fertilized by an X sperm likewise carrying the genes for yellow and white. Elimination at the first segmentation division of a maternal or of a paternal X, gave a cell with a single X, from which is descended the male right side.



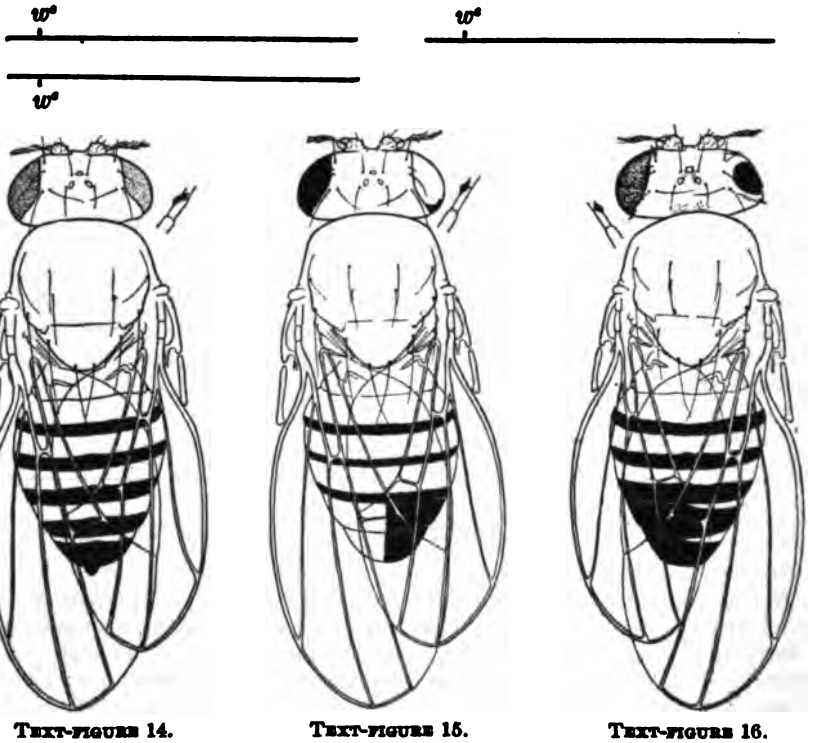
No. 77. March 10, 1914. C. B. Bridges. Text-figure 14 (diagram).

Parentage.—The mother was homozygous for eosin and heterozygous for a non-sex-linked gene "cream," which is a specific dilutor for eosin (Bridges, 1916). The father had the same constitution.

Description.—The gynandromorph was completely bilateral "from head to tail." The right side was male with a sex-comb and smaller parts. The abdomen had the female coloration above, but below was divided half and half, as were the genitalia. The eyes were both "cream," that is, of diluted

eosin color. The right male eye was much lighter than the female left eye, as is the rule with eosin even when diluted.

Explanation.—Both egg and sperm carried the genes for eosin and cream. Elimination of the paternal or of the maternal X occurred. Presumably the autosome carrying the cream gene behaved normally as in all known cases, but this case is not diagnostic, since the fly was homozygous for cream.



TEXT-FIGURE 14. TEXT-FIGURE 15. TEXT-FIGURE 16.

No. 1–5. A. M. Brown. April 9, 1914. Plate 2, Figure 4 (colored drawing).

Parentage.—The grandmother was homozygous for vermilion and heterozygous for a lethal. The grandfather was eosin-miniature. A gynandromorph was produced by one of their wild-type daughters which had been out-crossed to an eosin-miniature male.

Description.—The right side was male throughout with an eosin (male color) eye and miniature wing. A sex-comb was present on the right foreleg. The left side was entirely female, with red eyes and a long wing.

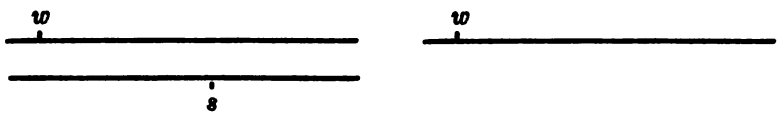
Explanation.—A non-cross-over egg containing the wild-type X was fertilized by an X sperm with genes for eosin and for miniature. Elimination of one of the maternal X's left the male parts eosin-miniature.

No. 195. April 27, 1914. C. B. Bridges. Text-figure 15 (drawing).

Parentage.—One X chromosome of the mother carried the gene for white eye-color and the other X the genes for eosin and for lethal 4. The X chromosome of the father carried the gene for sable.

Description.—The right side of the gynandromorph was male, with a white eye (with a fleck of red in it) and a sex-comb. The right wing was smaller. The genitalia were mainly male, but also with some female parts. Testes were found in sections of the abdomen, with plenty of sperm.

Explanation.—An egg containing the white X was fertilized by a sable-bearing X sperm. A paternal sperm suffered elimination, leaving the white-bearing X to produce the male side. The female side is wild-type, since one X has the normal allelomorph for white (viz, red eye), and the other X the normal allelomorph for sable (viz, wild-type body-color).

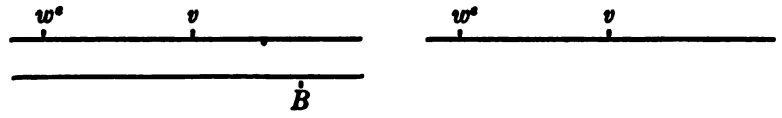


No. 1373. February 22, 1915. C. B. Bridges. Text-figure 16 (diagram).

Parentage.—One of the X chromosomes of the mother carried the genes for eosin and for vermilion, and the other X the genes for sable body-color and forked bristle. The X chromosome of the father carried the dominant gene for bar.

Description.—The left side of the gynandromorph was male throughout, being of smaller size in head, thorax, abdomen, wing, bristles, and legs, and having a sex-comb. The right eye was eosin-vermilion in color and not-bar. The coloration of the abdomen was male on the left side at the tip, and the genitalia were very largely male. The right side was female, with a red-bar eye. The abdomen was sectioned and found to contain a pair of poorly developed ovaries.

Explanation.—An egg containing the X chromosome with genes for eosin and for vermilion was fertilized by the bar-bearing X sperm. Elimination of a paternal X chromosome left the eosin-vermilion-bearing X to produce the male side, while the female side contained the dominant allelomorphs for these two genes, as well as the dominant gene for bar eye in the female XX complex.



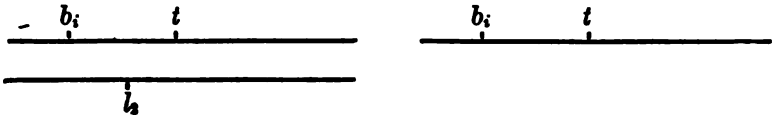
No. D. From Lethal 2 Stock. May 1, 1915. E. M. Wallace. Text-figure 17 (diagram).

Parentage.—The wild-type mother carried lethal 2 in one X and the genes for bifid and tan in the other. The stock was maintained by repeating in each generation the cross of wild-type lethal-bearing females to their bifid-tan brothers.

Description.—The gynandromorph was completely bilateral, the left side being male and the right female. The left side showed tan body-color throughout and had a bifid wing. The left side showed all the size, coloration, and other secondary sexual characters of the male. The genitalia were male. Sections showed that two rudimentary ovaries were present.

Explanation.—A non-cross-over egg containing the genes for lethal 2 was fertilized by an X sperm with the genes for bifid and tan. Elimination of

one of the maternal lethal-bearing X's occurred with the production of bifid tan male parts.



No. 1818. July 4, 1915. C. B. Bridges. Text-figure 18 (diagram).

Parentage.—One X chromosome of the mother carried the genes for sable and for forked; the other was wild-type. The X chromosome of the father carried the gene for forked.

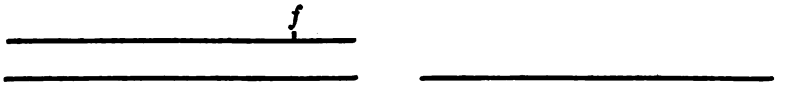
Description.—The gynandromorph was completely bilateral, the left side being male and the right female. The fly was not forked on either side. The genitalia were female. Sections showed ovaries on both sides.

TEXT-FIGURE 17.

TEXT-FIGURE 18.

TEXT-FIGURE 19.

Explanation.—An egg containing the normal X chromosome was fertilized by the forked sperm. A paternal X suffered elimination, leaving a normal X to produce the male side. The female side was also normal, because the maternal X present carried the normal allelomorph of forked.



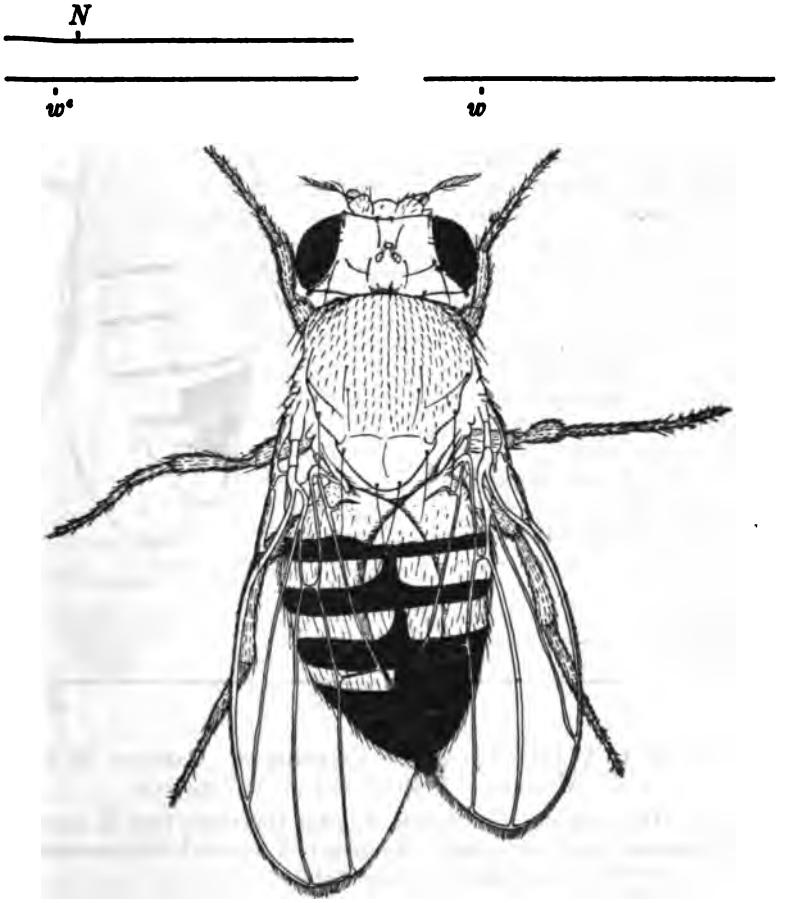
No. T. July, 1915. E. M. Wallace. Text-figure 19 (diagram).

Parentage.—The parentage is unrecorded, but from the characters shown by the gynandromorph it is probable that the fly came from notch stock. The mother carried the dominant gene for notch wing in one X and the gene for eosin in the other. The father was eosin.

Description.—The right side was male with a sex-comb, a short wild-type wing, smaller bristles, smaller half-thorax and half-abdomen. The tip of

the abdomen had male coloration on both sides and the genitalia were largely male. The right eye was eosin of the male type. The left side was mostly female with a red eye and a large notched wing. The gonads as seen through the body-wall seemed to be both ovaries.

Explanation.—A notch-bearing egg was fertilized by an eosin sperm. Elimination of the maternal notch X occurred.



TEXT-FIGURE 20.

No. F. January, 1916. T. H. Morgan. Text-figure 20 (diagram).

Parentage.—The parentage of gynandromorph F is unrecorded, though it is probable that it was found in a wild stock.

Description.—The fly was a completely bilateral gynandromorph having on the right side a sex-comb, shorter wing, shorter bristles, and smaller parts in head, thorax, and abdomen. The coloration of the abdomen was male at the tip on the right side, but female in the remainder. The genitalia were entirely female. The abdomen contained a fully developed pair of ovaries and she produced many offspring which were all wild-type.

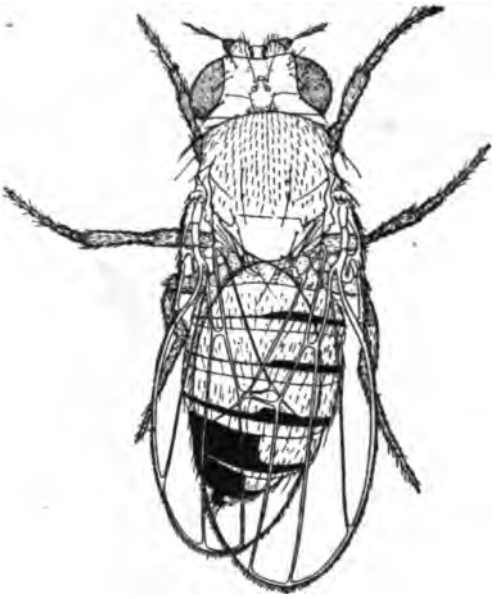
Explanation.—Elimination of one X occurred in a normal female zygote; whether this X was maternal or paternal is indeterminable.

No. 380. March 28, 1916. A. Weinstein. No diagram.

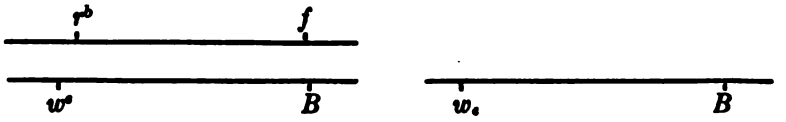
Parentage.—The mother carried the genes for ruby and forked in one X and the genes for eosin and sable in the other X. The father was eosin-bar.

Description.—The gynandromorph was about half and half, the left side being mainly male and the right female. The left side of the head and thorax and the left wing were smaller and the left foreleg bore a partly double sex-comb. The left eye was eosin bar of the male type. The genitalia were double posteriorly; there was a penis with claspers and anterior to the right of this an ovipositor and female-type anal prominences. The abdomen was female in coloration, except at the tip on the left side, which showed the male banding. The right eye was red and of the broad heterozygous bar female type.

Explanation.—A ruby forked X egg was fertilized by an eosin bar X sperm. Elimination of the maternal ruby forked X occurred.



TEXT-FIGURE 21.

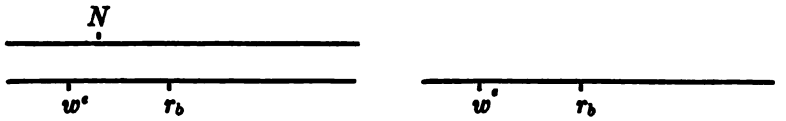


No. SSO1122AAA7344512 Selection Experiment. January 18, 1917. T. H. Morgan. Plate 4, Figure 1 (diagram).

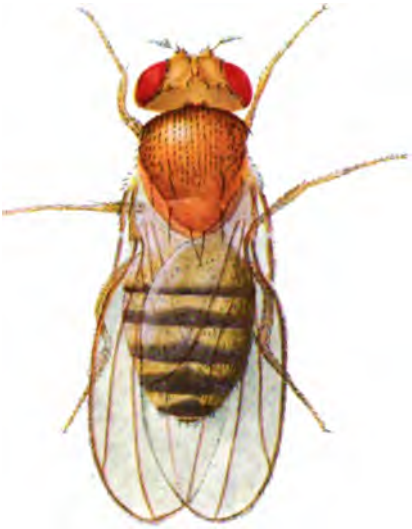
Parentage.—The mother was notch, having therefore one X chromosome with the dominant gene for notch; the other X carried the recessives eosin and ruby. The father was likewise eosin ruby.

Description.—The gynandromorph was male on the right side, except for spots of red (female) in the eosin ruby eye of that side. The coloration of the abdomen was male throughout. The genitalia were mainly male, but showed female parts. The left side was mainly female, having a red eye and a notch wing of slight type. No gonads were found in the sections examined, but it is probable that there were very rudimentary ovaries.

Explanation.—An egg bearing the gene for notch was fertilized by an X sperm with the genes for eosin and for ruby. Elimination of a maternal X chromosome left the male parts to be determined by the paternal eosin ruby X.



1



2

1a

3. Normal ♀

3a. Normal ♂

2a

4

5

E. M. Wallace Pinx

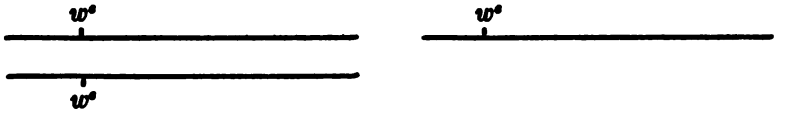
GYNANDROMORPHS OF DROSOPHILA

No. 29. February 11, 1918. T. H. Morgan. Text-figure 21 (drawing).

Parentage.—Both mother and father were eosin.

Description.—The gynandromorph was bilateral, except that the entire head was female, having eosin eyes of the dark homozygous eosin color. The left side was male, having a sex-comb, shorter legs, shorter bristles and wing, and a smaller left side to the thorax and abdomen. The coloration of the abdomen was half and half, but there appeared to be a pair of ovaries and female genitalia.

Explanation.—An egg containing an X chromosome with the gene for eosin was fertilized by an X sperm carrying eosin. Elimination of either X gave the nearly bilateral gynandromorph.



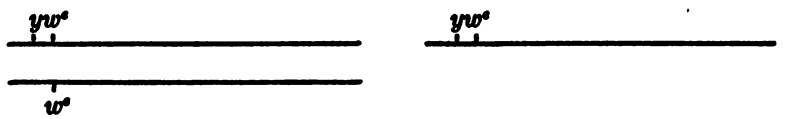
GYNANDROMORPHS MAINLY FEMALE.

No. C_2C_{19}. January 1914. E. M. Wallace. Plate 2, Figure 3 (colored drawing).

Parentage.—The mother was white-eosin compound, having the genes for yellow and white in one X and eosin in the other X. The father was eosin.

Description.—All of the fly was gray and female, except the upper right half of the thorax and the right wing, which were yellow and male. Both eyes were eosin, of the dark type of the homozygous eosin female. Well-developed ovaries were present on both sides. Mated to a yellow white male this gynandromorph was fertile and produced white-eosin females 70; eosin males 42; yellow white-eosin females 53; yellow eosin males 58.

Explanation.—An egg with a cross-over X containing the genes for yellow and eosin was fertilized by an X sperm with a gene for eosin. Elimination of one of the latter left the maternal X to produce the male parts on the upper right side of the thorax.



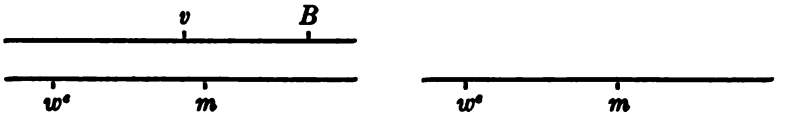
No. 5137. September 4, 1916. C. B. Bridges. Plate 2, Figures 5 and 5*a* (colored drawings).

Parentage.—The mother had one chromosome with the genes for vermilion, sable, garnet, and forked, and the other X with the genes for vermilion and for bar. The X-bearing sperm carried the genes for eosin and for miniature wings.

Description.—The mosaic was entirely female, except for a patch of eosin in the left eye. The eosin part of the eye was round and light eosin (male type) while red bar both above and below (very slight amount below). The right eye was red bar. The whole abdomen was full of eggs.

Explanation.—An egg with the X chromosome carrying the genes for vermilion and bar was fertilized by a sperm carrying the genes for eosin and miniature. Elimination of a maternal chromosome took place, leaving the

one X with eosin and miniature genes to produce the male parts, which in this case affected visibly only a part of the left eye.



No. M, 114. January 23, 1914. C. B. Bridges. Text-figure 22 (diagram).

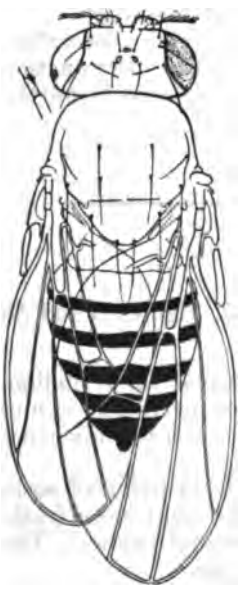
Parentage.—One of the X chromosomes of the mother contained the gene for eosin, the other the gene for bar. The father was white bar. Both parents were heterozygous for the autosomal recessive gene "whiting," which is a specific modifier of eosin.

Description.—The gynandromorph was somewhat more than half female. The left side of the gynandromorph, except for the head, was male, with sex-comb, smaller bristles half-thorax and wing. In coloration the abdomen was male on the left and female on the right. The genitalia were entirely female. The head had heterozygous bar (female) eyes, which were white-eosin compound (female) in color. A pair of ovaries was present.

Explanation.—An egg containing the X chromosome with the gene for eosin was fertilized by the X sperm with the genes for white and for bar. Either chromosome may have been the one to suffer elimination; which one it was could not be determined, since the head did not show male parts.

TEXT-FIGURE 22.

No. 438. August 16, 1914. C. B. Bridges. Text-figure 23 (diagram).

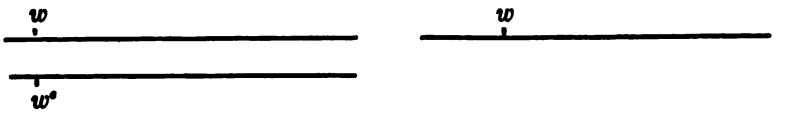


TEXT-FIGURE 23.

Parentage.—One X chromosome of the mother contained the genes for eosin and for vermilion, the other X the gene for white eyes. The X chromosome of the male carried the gene for eosin.

Description.—The left side of the gynandromorph was largely male with a white eye (containing a fleck of white-eosin), a sex-comb, and a shorter wing. The right side was female, with a white-eosin compound eye, no sex-comb, and a longer wing. The abdomen was banded like a female. When bred as a female the fly gave the classes expected for a white-eosin compound. No sections were made.

Explanation.—An egg containing the X chromosome with the gene for white was fertilized by an X sperm carrying the gene for eosin. The latter—the paternal chromosome—suffered elimination, leaving the white-bearing X to produce the male side. The color of the eye on the female side was white-eosin compound, which is the expected result for the two X's involved.

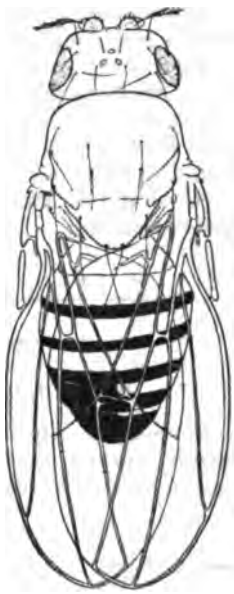


No. 922. December 16, 1914. C. B. Bridges. Text-figure 24 (diagram).

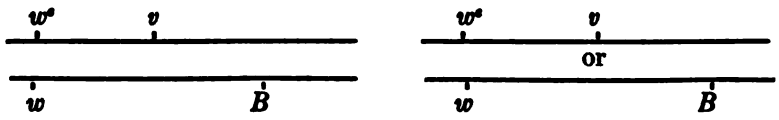
Parentage.—One of the X chromosomes of the mother contained the genes for eosin and for vermilion, the other X the gene for forked. The X chromosome of the male carried the genes for white and for bar.

Description.—The fly was female throughout (without sex-combs) and possessed white-eosin heterozygous-bar eyes, except that the tip of the abdomen on the left side was banded like a male. Below there was a normal penis and male armature. In sections an ovary was found on one side, nothing on the other.

Explanation.—An egg containing the X chromosome with the genes for eosin and for vermilion was fertilized by the X-bearing sperm with the genes for white and for bar. Elimination of either X chromosome would account for the male parts at the tip of the abdomen.



TEXT-FIGURE 24.

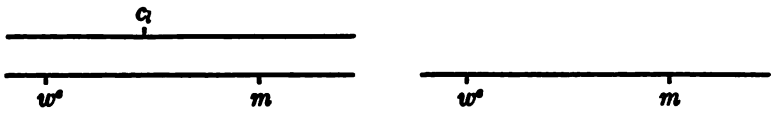


No. 925. December 18, 1914. C. B. Bridges. Text-figure 25 (diagram).

Parentage.—The mother was club, carrying in one X the sex-linked gene club, and in the other X lethal 2 which is a deficiency for club. The X sperm of the father carried the genes for eosin and for miniature.

Description.—The only male part was the right eye, which was eosin (male type) in color, except for a fleck of red (female). The fly was fertile as a female when mated to a wild male and produced: No. 1117; wild type females, 101; eosin miniature males, 55; miniature male, 1; eosin males, 9.

Explanation.—An egg with an X bearing the gene for lethal 2 was fertilized by an X sperm with the genes for eosin and miniature. Elimination took place in one of the maternal chromosomes, leaving the paternal X with eosin and miniature to form the male parts, viz, the right side of the head (in part). The rest of the mosaic was female; hence both wings were wild-type.

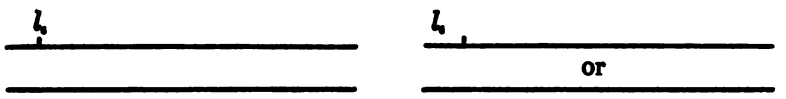


No. 1010. December 20, 1914. C. B. Bridges. Text-figure 26 (diagram).

Parentage.—One X chromosome of the mother carried the genes for yellow and for white, and the other X the gene for lethal 6. The X chromosome of the father carried only wild-type genes.

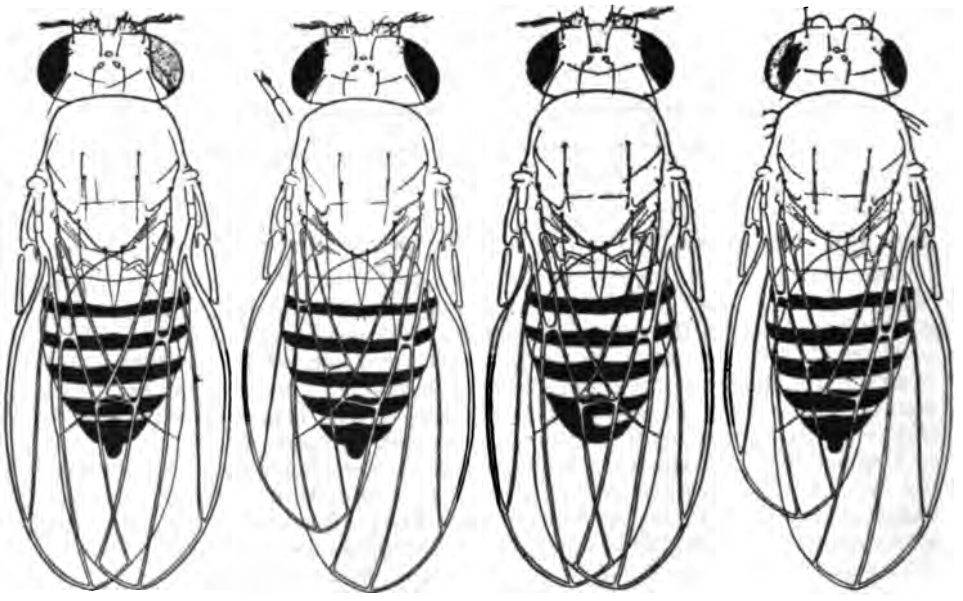
Description.—The left side of the thorax of the gynandromorph was male, with a sex-comb and a shorter wing. Both eyes were red and female. The abdomen and genitalia were female. The body-color was wild-type throughout. Sections showed ovaries on both sides.

Explanation.—An egg containing the lethal 6 X chromosome was fertilized by a wild-type X sperm. Either one of the X chromosomes being eliminated would account for the result. If the paternal X were eliminated the male parts would be lethal 6, and hence it is more probable that the maternal X was eliminated.



No. 1808. July 7, 1915. C. B. Bridges. Text-figure 27 (diagram).

Parentage.—The mother was pure for the second-chromosome recessives purple, curved, and speck. The father was heterozygous for the dominant star (eyes). No sex-linked mutant characters were present.



TEXT-FIGURE 25. TEXT-FIGURE 26. TEXT-FIGURE 27. TEXT-FIGURE 28.

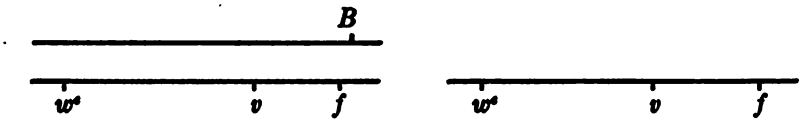
Description.—The gynandromorph was female throughout, except for the abdomen, which had male coloration on the left side and was twisted to the left. A perfect penis was present. The eyes were star. The male parts could not have shown the recessive second-chromosome characters, even had they been present. No testes or ovaries were found, but there was a genital tube with pointed cells like abnormal spermatozoa.

No. 5238. September 23, 1916. C. B. Bridges. Text-figure 28 (diagram).

Parentage.—One of the X chromosomes of the mother carried the genes for vermilion eye-color and for bar eye, the other X the gene for forked bristles. The X chromosome of the father carried the genes for eosin, vermilion, and forked.

Description.—The fly was mainly female, but is exceptionally interesting from the peculiar description of the male parts, which constitute a very narrow stripe running through the middle of the left eye and along the left side of the thorax, including the wing. The left eye was eosin vermilion in color in the male parts and red in the female parts, both above and below the eosin vermilion. These female parts were heterozygous for bar and the red portions above and below were therefore characteristically narrow, while the eosin-vermilion part was not-bar and projected forward, so that the male stripe could be traced forward to the normal margin of the round eye. The male part of the thorax could likewise be traced by means of the forked bristles, of which there were three anterior to the wing, one above, and none below. The wing itself was included in the male region and was smaller and had forked marginal bristles. There was no sex-comb on the left side.

Explanation.—An egg containing an X chromosome with the gene for bar was fertilized by the eosin vermilion forked sperm. A maternal X suffered elimination, leaving the eosin vermilion forked X to produce the male parts.



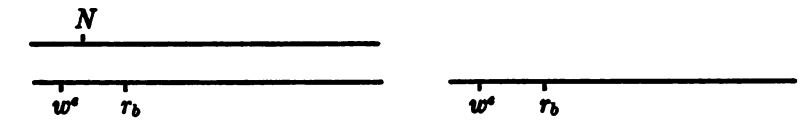
No. 2. September, 1917. T. H. Morgan. Text-figure 29 (drawing).

Parentage.—The fly appeared in "selected notch" stock in which, in each generation, red-eyed notch females were bred to eosin ruby males.

Description.—The right eye was red, the left partly red, partly eosin ruby, with a very irregular boundary-line; otherwise the fly was female.

Explanation.—An egg with a gene for notch wing was fertilized by an X sperm bearing eosin and ruby. Elimination of one of the maternal X's left a part of one side of the head with the eosin ruby X. The wings, although not showing notch, must have contained the gene. Since less than half of the notch flies in this selected stock showed the notch character, its absence here is not difficult to explain.

TEXT-FIGURE 29.

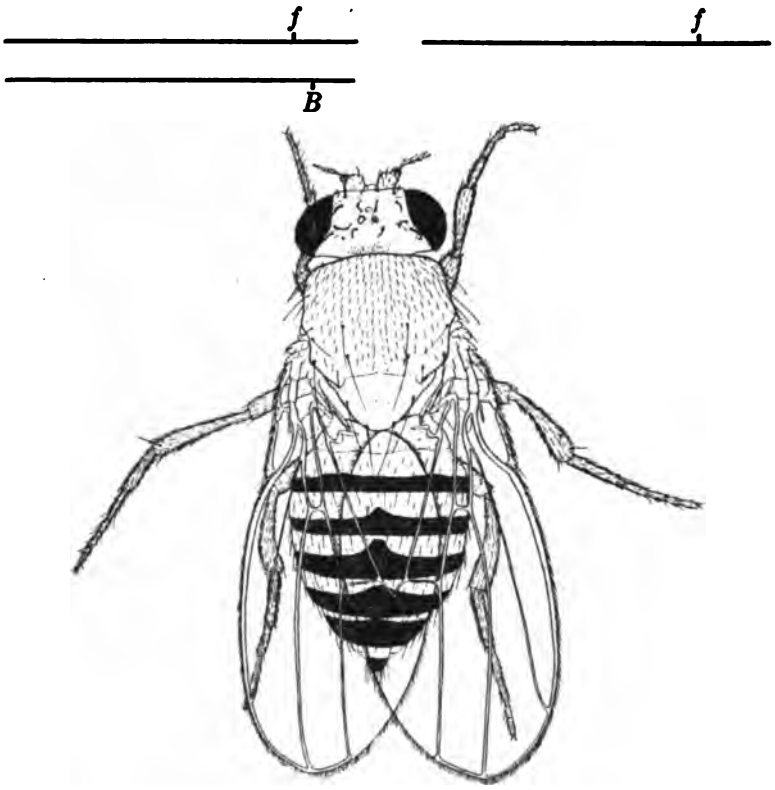


No. 477. October 31, 1917. D. E. Lancefield. Text-figure 30 (drawing).

Parentage.—One of the X chromosomes of the mother had a bar gene, the other a gene for forked. The father was bar.

Description.—The head was small, with round eyes and forked bristles. The thorax and wings seemed to be female. No sex-combs present. The abdomen was entirely female, with eggs inside, but she did not breed.

Explanation.—An egg containing an X with a gene for forked was fertilized by a bar X sperm. The paternal X with bar was eliminated, leaving the head male and forked.



TEXT-FIGURE 30.

No. 71. October 23, 1917. E. M. Wallace. Text-figure 31 (drawing).

Parentage.—Pure stock of bar.

Description.—A female was observed that had a short left wing. Closer examination showed that the bristles on that side of the thorax and head were shorter and that the left side of the head was slightly contracted and the eye smaller. It is probable that the left side of head and thorax (dorsally) were male.

No. 7530. August 18, 1917. C. B. Bridges. Text-figure 32 (diagram).

Parentage.—One of the X chromosomes of the mother carried the gene for facet eye and the other X the gene for notch wings (dominant). The X chromosome of the father carried the gene for facet.

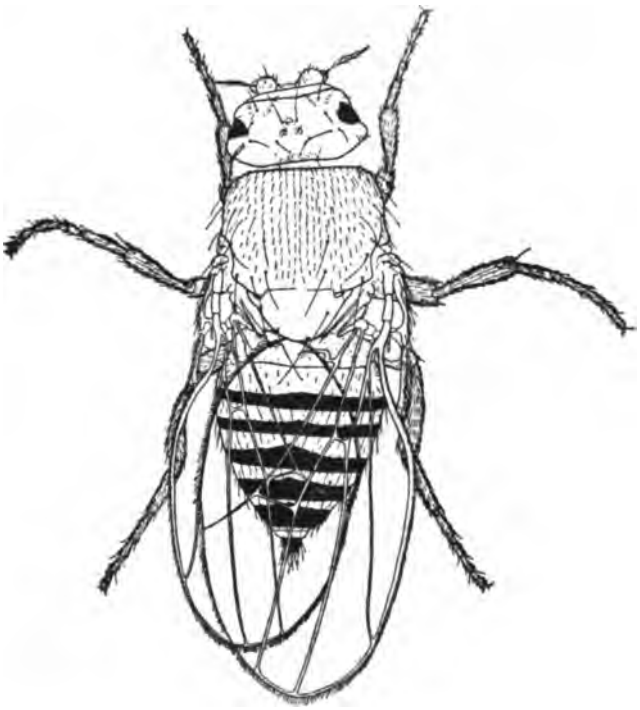
Description.—The left side of the gynandromorph was male, with a shorter wing, sex-comb, and smaller eye, whose markedly faceted eye was characteristic for that character as it appears in the male of the mutant type. The female side had a faceted eye of the female type, which is far less marked. The abdomen was banded as in the female, but below a penis was present. Testes were found on both sides, with an abundance of sperm.

Explanation.—An X egg-carrying facet was fertilized by the X sperm-carrying facet. Elimination of either occurred. The gonads were formed from a male cell. Very frequently a male-appearing abdomen contains ovaries; only very rarely does a female-type abdomen contain testes.

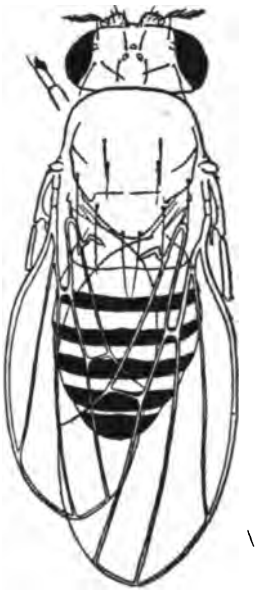
No. XI. January, 1914. E. M. Wallace. No diagram.

Parentage.—This gynandromorph arose in a mass-culture whose parents were yellow white females and eosin males.

Description.—The gynandromorph was largely female. The male parts were yellow and included the left dorsal side of the thorax with the shorter wing and the left side of the abdomen. These parts were all smaller, bore



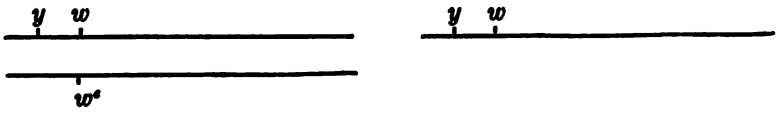
TEXT-FIGURE 31.



TEXT-FIGURE 32.

smaller bristles, and the left half of the abdomen had male-type coloration. The genitalia were female. The female parts throughout were wild-type in body-color, including especially the left legs and all the head. There were no sex-combs. The eyes were both white-eosin compound.

Explanation.—A yellow white X egg was fertilized by an eosin X sperm. Elimination of the paternal X occurred.



GYNANDROMORPHS MAINLY MALE.

No. $G_1Ab_2Ca_2$. March, 1914. E. M. Wallace. Plate 2, figures 6 and 6a (colored drawings).

Parentage.—The mother was a yellow white female, a daughter of gynandromorph G_1Ab_2C. The father was an ebony (third-chromosome) male. This mating was part of the second of the tests specifically designed to show the absence of elimination of autosomes in the production of gynandromorphs.

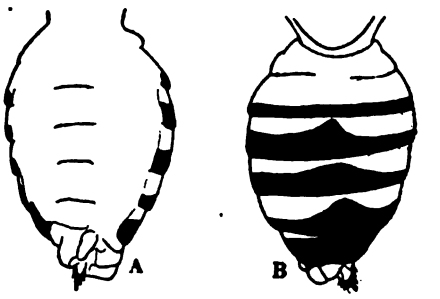
Description.—The gynandromorph was mainly male, with only the head and genitalia female. The color of the entire thorax, abdomen, legs, and wings was yellow, and correspondingly the bristles of these parts were brown. These yellow parts were male, as proved by the sex-combs on both forelegs, by the small (male) size of the bristles, of the thorax, and particularly of the abdomen, and by the male coloration and shape of the abdomen. However, the genitalia were an exception, for the anal prominence and the ovipositor were purely female in structure and bore black spines which showed that the body-color was wild-type. The head was entirely female, as proved by its large size, the wild-type color with black bristles, and by the red eyes. Thus the head and genitalia—the two ends of the fly—were female and all the region between was male.

Explanation.—An egg carrying the genes for yellow and white was fertilized by sperm carrying only wild-type genes in the X. Elimination of a paternal X occurred and subsequent shifting isolated a female cell which gave rise to the genitalia. The absence of ebony proves that the third chromosome did not undergo elimination.

No. X_2. February 1914. E. M. Wallace. Text-figure 33 (drawing).

Parentage.—Gynandromorph X_2 appeared in a mass-culture, the mothers of which carried yellow and white in one X and eosin in the other; the fathers were yellow-white.

Description.—The gynandromorph was mainly male. The female parts were confined to the abdomen, which had female coloration on the left side and apparently male on the right. The abdomen was twisted to the right, which also suggests that the right side was male. However, the genitalia reversed this relation, the right side being largely female, with an anal prominence of female type; the left side was male and there was a median penis. The abdomen was of large size and a pair of ovaries could be clearly seen within. The thorax and head were entirely male, as evidenced by their size and the type of bristles and the presence of sex-combs on both forelegs. The eyes were both white and the body-color was yellow throughout.



TEXT-FIGURE 33.

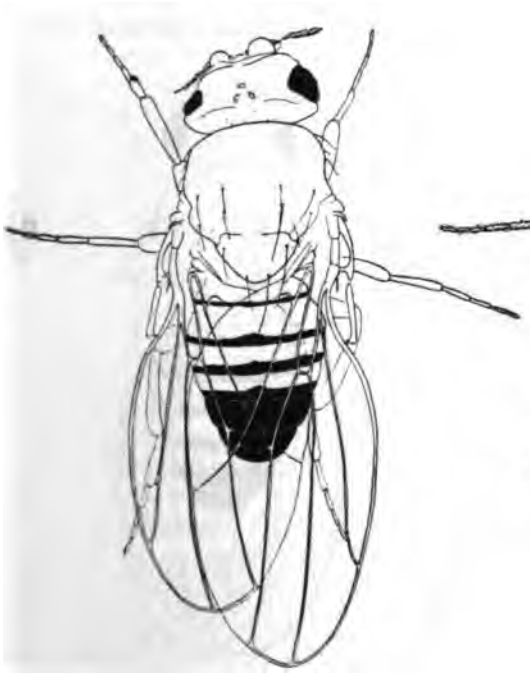
Explanation.—A yellow white X egg was fertilized by a yellow white X sperm. Elimination of either X occurred. An alternative explanation is that the egg was fertilized by a Y sperm giving a yellow white male. Somatic non-disjunction resulted in a cell with both daughter X's present, and this gave rise to the female parts.

No. 3. February 1915. T. H. Morgan. Text-figure 34 (drawing)

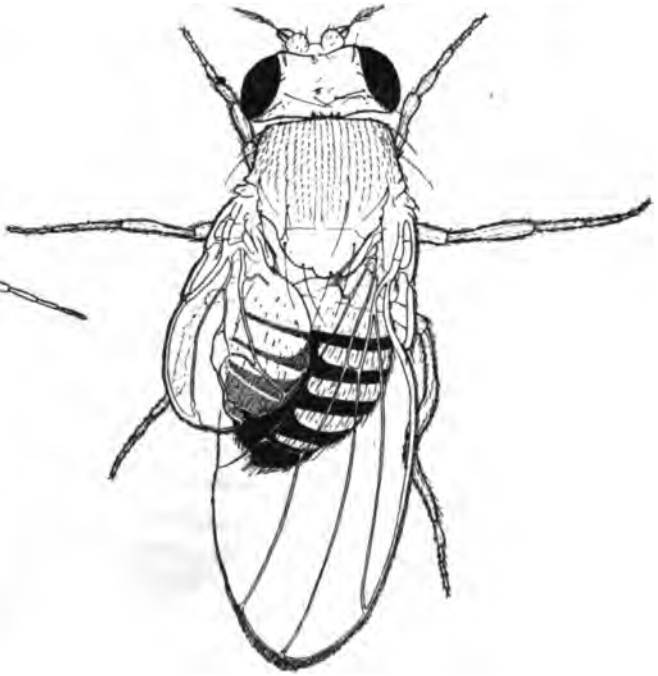
Parentage.—The mother was rudimentary and the father bar.

Description.—The gynandromorph was about three-fourths male. The right halves of the head and of the thorax were female, being larger in size, having larger bristles and a larger wing, which was wild-type, and no sex-comb. The right eye was heterozygous bar (female). The left eye was bar of the male type and the left halves of the head and of the thorax were male. The left wing was smaller, but not rudimentary. The abdomen seemed entirely male, with a normal penis. This gynandromorph was tested as to sexual behavior and was found to pay no attention to mature virgin females. An account of this gynandromorph and the drawing have been previously published. (Morgan, Am. Nat., V. 49, p. 240, April 1915.)

Explanations.—An egg with a rudimentary X was fertilized by an X sperm carrying bar. Elimination of the maternal rudimentary X occurred. Some of the female cells were lost in cleavage, so that the individual is preponderantly male.



TEXT-FIGURE 34.



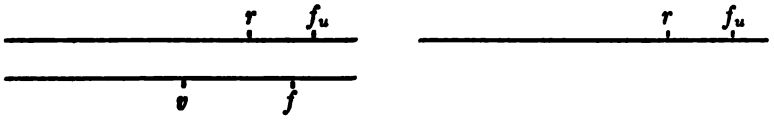
TEXT-FIGURE 35.

No. 2317. November 2, 1915. C. B. Bridges. Text-figure 35 (drawing).

Parentage.—One X chromosome of the mother carried the genes for rudimentary wing and fused veins, and the other X the gene for bar. The X chromosome of the father carried the genes for vermilion eye and forked bristles.

Description.—The left side of the gynandromorph is male, with sex-comb and rudimentary fused wing, the left side of the abdomen is male, but the genitalia are female. The ocelli on the head are like those of fused, and the head is therefore male. No sections were made.

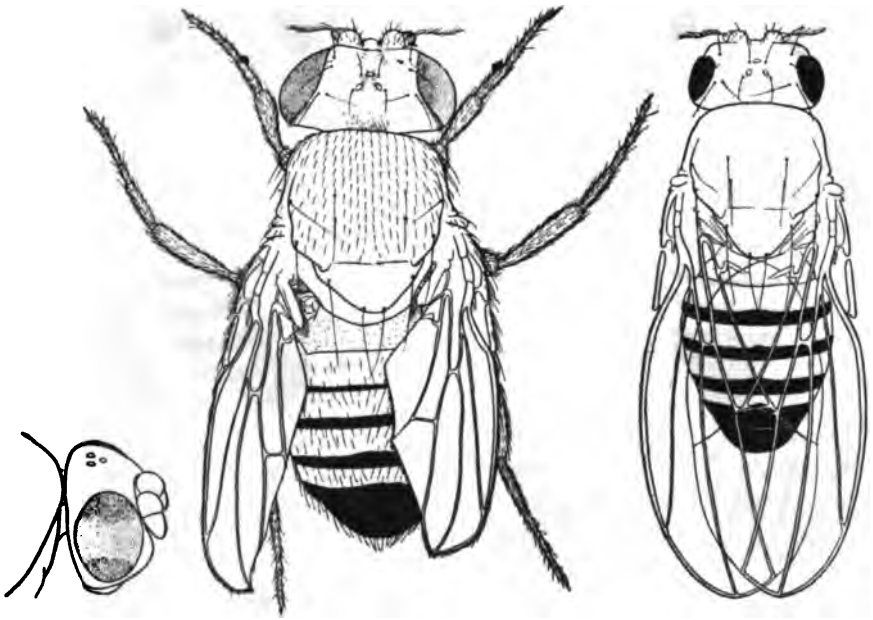
Explanations.—An egg containing the rudimentary fused X was fertilized by the vermilion forked sperm. A paternal vermilion forked X was eliminated, leaving the other rudimentary fused X to produce the male side, while the female side contains both original X's, namely, rudimentary fused and vermilion forked, and is accordingly wild-type.



No. 3272. February 10, 1916. C. B. Bridges. Text-figures 36 and 36*a* (drawings).

Parentage.—One X chromosome of the mother carried the genes for sable, garnet and also "sable-duplication" at zero. The other X carried the genes for eosin and for miniature. The father was eosin-miniature.

Descriptions.—The entire abdomen was apparently male in shape, banding, and genitalia, though it is not known whether testes or ovaries were present. The right side of the thorax was smaller in size and bore smaller bristles and



TEXT-FIGURE 36*a*. TEXT-FIGURE 36. TEXT-FIGURE 37.

a smaller (male-type) miniature wing. All right legs were male and both forelegs bore sex-combs. The right eye (see drawing) had a streak of male tissue ("light" eosin color) running forward completely through. Above and below this streak the tissue was female ("dark" eosin color) There is one other curious feature—the left foreleg as well as the right bore a sex-comb. The head, except the male streak, the right side of the thorax with its miniature wing, and the two rear legs were female

Explanations —An egg bearing the eosin miniature non-cross-over X was fertilized by the X sperm carrying eosin and miniature Elimination of one of these X's (either maternal or paternal) was followed by shifting of cleavage nuclei or by shifting of the anlage in the formation of the pupa.

GYNANDROMORPHS ROUGHLY "FORE-AND-AFT."

No. II 139. January 12, 1914. C. B. Bridges. Text-figure 37 (diagram).

Parentage.—The mother was black (second chromosome), but carried only wild-type genes in her X chromosomes. The father was a bar not-black male.

Descriptions.—The fly was heterozygous bar in both eyes and female throughout, except for the external genitalia, which were male (penis), and the coloration of abdomen. Sections showed that a pair of ovaries was present.

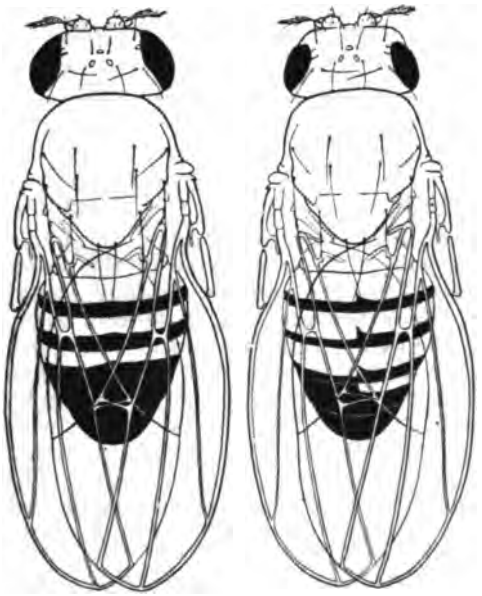
Explanations.—An egg with a wild-type X was fertilized by the X sperm with the gene for bar. Since the male parts did not involve the eye, it can not be determined whether they arose from cells carrying the bar (paternal) or the wild-type (maternal) X. The fly did not show black in the male parts, but since the male region was so small and also normally dark-colored, this case could not be accepted as proving that the elimination did not affect the autosomes, as is proved in several later cases, especially devised for that purpose.

$$\frac{\quad}{\quad}\Big/\frac{\quad}{B} \qquad \text{or} \qquad \frac{\quad}{\quad}\Big/\frac{\quad}{B}$$

No. 1813. July 5, 1915. C. B. Bridges. Text-figure 38 (diagram).

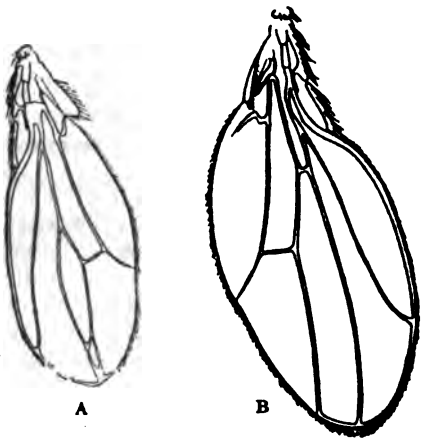
Parentage.—One X chromosome of mother carried the genes for forked and for cleft (wing); the other X only wild-type genes. The father was forked.

Descriptions.—The head, thorax, wings, and legs were female. The abdomen had the male coloration and a normal penis. The (poor) sections



TEXT-FIGURE 38.

TEXT-FIGURE 39.



TEXT-FIGURE 40.

showed that at least one ovary was present. The wings were not cleft and the male parts showed no forked spines.

Explanations.—The egg contained the wild-type X and was fertilized by the X sperm carrying forked. The paternal X was eliminated, leaving the male parts wild-type.

$$\frac{\quad}{f}\Big/\frac{\quad}{\quad} \qquad \frac{\quad}{\quad}$$

No. 2204. October 5, 1914. C. B. Bridges. Text-figure 39 (diagram).

Parentage.—One X of the mother carried the gene for eosin, the other the genes for vermilion and forked. The father was bar.

Description.—The gynandromorph was of the "fore-and-aft" type. The abdomen was of the male shape, with male coloration on the left side and partially male on the right. There was a normal penis. The eyes were heterozygous bar (female) and the head, thorax, legs, and wing were female. Sections showed that small ovaries were present.

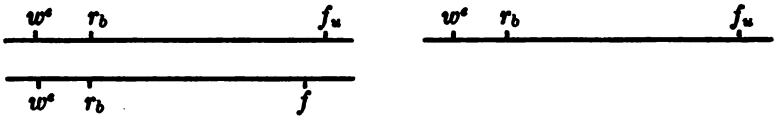
Explanations.—Since the male parts were not forked, the egg probably carried the eosin X. The X sperm carried bar. The eyes were female and there is no criterion as to which X was eliminated. An alternative explanation assumes a vermilion forked X in the egg, and subsequent elimination of this same X to give the not-forked male parts.

No. X. August 1916. A. Weinstein. Text-figure 40 (drawings of wings).

Parentage.—The mother had the genes for eosin, ruby, and forked in one X and for fused in the other. The father was probably eosin ruby forked.

Description.—The gynandromorph was largely male anteriorly and female posteriorly. The head was entirely male, with eosin ruby eyes. There were sex-combs on both forelegs, which means that the ventral part of the thorax was male. The left dorsal part was also male, having a small wing which was fused. The right dorsal part was female with a large wild-type wing. The abdomen and genitalia were female.

Explanations.—The egg carried a cross-over eosin ruby fused X and the sperm an eosin ruby forked X. Elimination of the paternal X occurred.

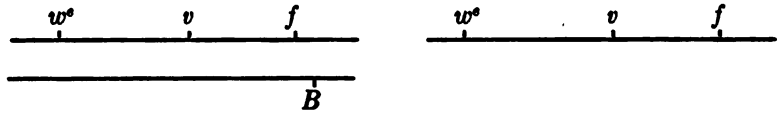


No. 4614. January 22, 1918. A. H. Sturtevant. Text-figure 41 (diagram).

Parentage.—One X of the mother carried the genes for eosin, vermilion, and forked; the other X carried only wild-type genes. One of the third-chromosomes carried the recessive genes for sepia, spineless, kidney, sooty, and rough; the other was wild-type. The father was a bar male from stock.

Description.—Except for the wings, the gynandromorph is divided antero-posteriorly. The right wing was slightly larger than the left and may have been female. The other wing and the remainder of the thorax was male. There were sex-combs on both forelegs. The head was entirely male, with eosin-vermilion eyes and forked bristles. The thorax and legs had also forked bristles. The abdomen was female, both in banding and in shape. The genitalia were female, but slightly abnormal. Tested as a female she proved sterile. None of the third-chromosome recessives showed in any part, either male or female, of the gynandromorphs.

Explanations.—An egg containing the non-cross-over eosin vermilion forked X was fertilized by an X sperm carrying bar. The paternal X was eliminated, producing the anterior male parts. The absence of the recessive third-chromosome characters in the male parts proves that the elimination of the X was independent of the third chromosome.

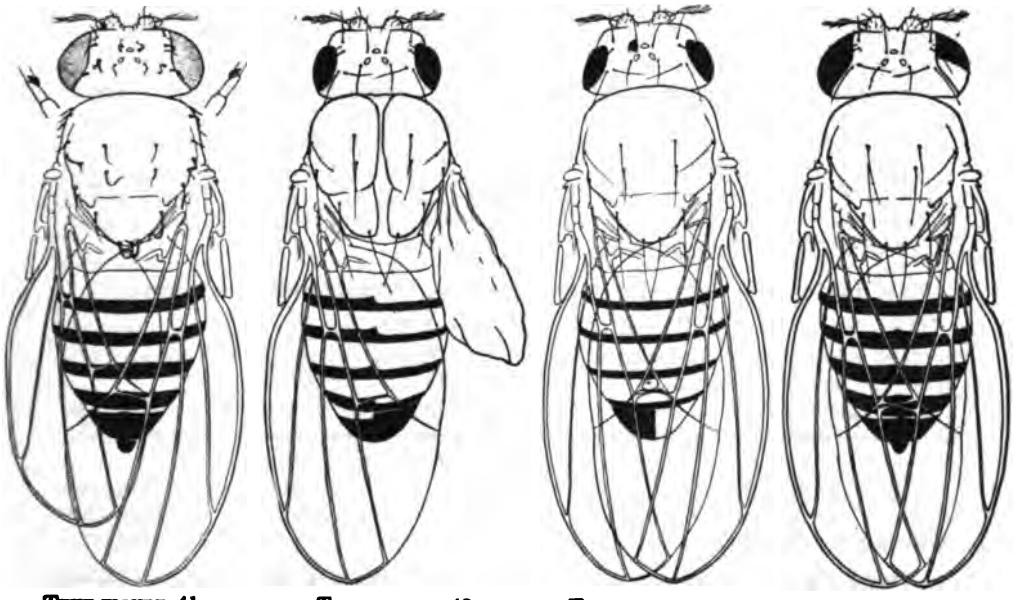


GYNANDROMORPHS PRODUCED BY XXY FEMALES.

No. N 2. December 12, 1912. C. B. Bridges. Plate 3, Figures 1 and 1*a* (colored drawings).

Parentage.—The mother was an XXY female homozygous for white and heterozygous for the third-chromosome mutant pink. The father was red-eyed, and also heterozygous for pink. Both parents were exceptions produced by secondary non-disjunction.

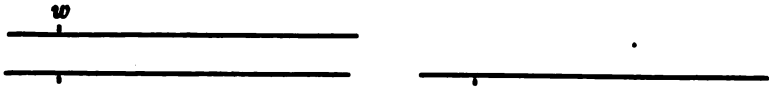
Description.—The fly was a completely bilateral gynandromorph, male on left side, female on right. The male side was smaller, with sex-comb, the genitalia half and half. The fly was unable to breed as a male or as a



TEXT-FIGURE 41. TEXT-FIGURE 42. TEXT-FIGURE 43. TEXT-FIGURE 44.

female. The abdomen was large and evidently contained a pair of ovaries. The fly was figured in Heredity and Sex, page 163, and the origin given in Journ. Exp. Zool., 1913, page 597.

Explanations.—A regular X egg carrying the gene for white was fertilized by an X sperm carrying the wild-type allelomorph red. One of the maternal X's, bearing the gene for white eye, was eliminated. The white-eye character therefore does not appear on either side. As both parents were heterozygous for pink, the fly may have come from third chromosomes bearing normal genes only, or one of them may have had the gene for pink, so that the gynandromorph is heterozygous.



No. N 3. November 30, 1912. C. B. Bridges. Plate 3, Figures 2 and 2*a* (colored drawings). (See fig. 17.)

Parentage.—The mother was an XXY female, carrying white in both X chromosomes. The father was a wild male.

Description.—The gynandromorph was entirely female, except for the tip of the abdomen below, where a perfectly normal penis and male genitalia were found. The anal prominence and the parts immediately surrounding the genitalia were also male. The posterior ventral plate was male type, being broad, rounded, and hairless.

No. 1221. February 2, 1915. C. B. Bridges. Text-figure 42 (diagram).

Parentage.—The mother was an XXY wild-type female, one of whose X chromosomes carried the gene for eosin, the other X only wild-type genes. The father was bar.

Description.—The gynandromorph was bilateral, except for the head, which was entirely female, with red bar eyes of the heterozygous type. The right side of the thorax dorsally was male, with shorter bristles and very small wing (abnormal). There were no sex-combs. The right side of the abdomen was male in coloration, and the genitalia were almost entirely male. There was a pair of testes with ripe spermatozoa. The two halves of the thorax failed to come together and the male and female parts were unfused.

Explanations.—The egg carried the eosin X and may or may not have contained a Y. The sperm was the X sperm carrying bar. Elimination of either X occurred. It is possible that the spina bifida condition may have been a result of the gynandromorphism.

No. 1892. July 19, 1915. C. B. Bridges. Text-figure 43 (diagram).

Parentage.—The mother was a wild-type XXY female, which was an exception from "high" non-disjunction. One X carried the gene for eosin, the other the genes for vermilion and forked. The father was bar.

Description.—The fly was female throughout, except that the left side of the abdomen, especially at the tip, showed male coloration and the genitalia were entirely male. The eyes were heterozygous bar (female).

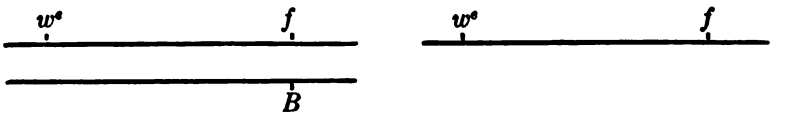
Explanation.—An egg with one X (either) and with or without a Y (even chance) was fertilized by an X sperm carrying the gene for bar. Elimination of either X occurred.

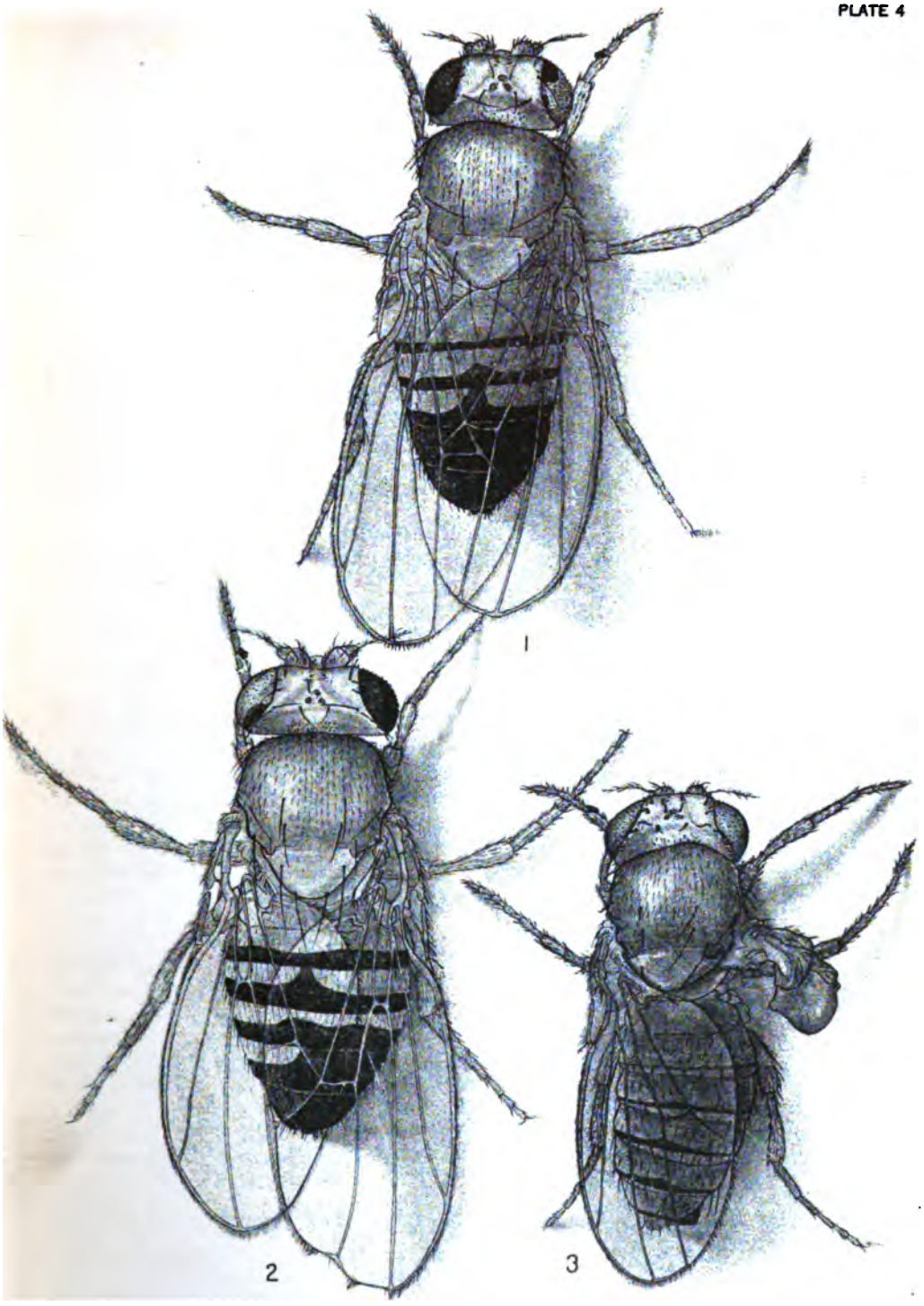
No. 7673. October 16, 1917. C. B. Bridges. Plate 4, Figure 3 (drawing).

Parentage.—The mother was an eosin-eyed XXY exception from a special strain of "high" non-disjunction (H_e), which had arisen by equational non-disjunction from the regular high strain. One of her X chromosomes carried the gene for eosin and the other the genes for eosin and forked. The father was bar.

Description.—The male parts of the gynandromorph constituted the entire head, which had eosin eyes of the male type and forked bristles; the left side of the thorax, which had forked bristles, was smaller, with a male-size wing and a sex-comb and a slight patch of male tissue at the tip of the abdomen, but on the right side, not the left. The abdomen was twisted, as it usually is when bilateral, but since the bristles were not forked, the male parts, if any, must have been below or internal. The right wing was abnormal.

Explanations.—An X egg carrying the genes for eosin and forked was fertilized by the X sperm carrying the gene for bar. Whether or not a Y was present in the egg is not known (chances even). Elimination of a paternal bar X occurred.





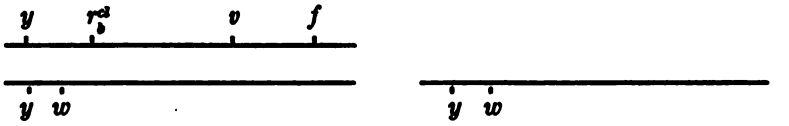
GYNANDROMORPHS OF DROSOPHILA

No. 5485. October 18, 1916. C. B. Bridges. Text-figure 44 (diagram).

Parentage.—The mother was an XXY female, one of whose chromosomes contained the genes for yellow and for white, the other X the gene for lethal 7. The X chromosome of the father carried the genes for yellow, claret, vermilion, and forked.

Description.—The gynandromorph was a yellow female, except that three-quarters of the right eye was white in color and male, the remainder, which was a perfect quarter sector of the eye, being red and female. Sections showed normal ovaries to be present.

Explanations.—An egg containing the X chromosome with the genes for yellow and for white was fertilized by the X sperm with the genes for yellow and the three other recessive genes named above. Elimination of a paternal chromosome occurred, leaving the yellow white X to determine the character of the male parts, viz, the right eye, except for a triangular area of female tissue.



GYNANDROMORPHS OF COMPLEX TYPE.

No. 487. November 27, 1917. D. E. Lancefield. Text-figure 45 (drawing).

Parentage.—The mother was an XXY female homozygous for eosin and miniature. The father was a wild male.

Description.—The distribution of male and female parts was very complex. The entire head was female, as evidenced by its large size and by the color of both eyes, which was eosin of the dark female type. The right dorsal part of the thorax was female, as shown by its large size and the large size of the bristles and of the wing, which was also wild-type and not miniature. The only other female parts seemed to be the left ventral part of the thorax, including left legs, since left foreleg carried no sex-comb. The other two sectors of the thorax—the right ventral and the left dorsal—were male, as proved by the smaller size of the parts themselves and of their bristles, and even better by the presence of a sex-comb upon the right foreleg and of a miniature wing of male size upon the left side. As the head was entirely female, the abdomen seemed to be entirely male, except that the armature seemed slightly different in the two sides of the penis.

Explanations.—An egg carrying an eosin miniature X (whether or not a Y also is unknown) was fertilized by the X sperm carrying only wild-type genes. Elimination of a paternal X occurred. The segmentation nuclei descended from this same pair of male and female cells were distributed in a regular but complex pattern.

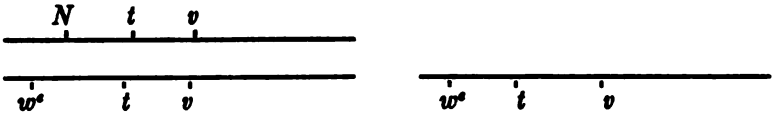
No. 941. December 15, 1914. C. B. Bridges. Text-figure 46 (diagram).

Parentage.—The parentage is somewhat uncertain, probably as follows: The mother had one X with eosin, notch, tan, and vermilion, and the other X wild-type. The father was eosin tan vermilion.

Description.—The gynandromorph was about half-and-half, but rather complex in the distribution of male and female parts. The head was large, therefore probably female. The eyes were alike and vermilion. The right wing was a typical notch (female) but was only doubtfully larger than the left. The abdomen was female in coloration anteriorly but male posteriorly.

The genitalia were largely male, but had female parts on the right side. A pair of rudimentary ovaries were present. There were sex-combs on both forelegs, so that the ventral side of the thorax was entirely male. The fly was tan throughout.

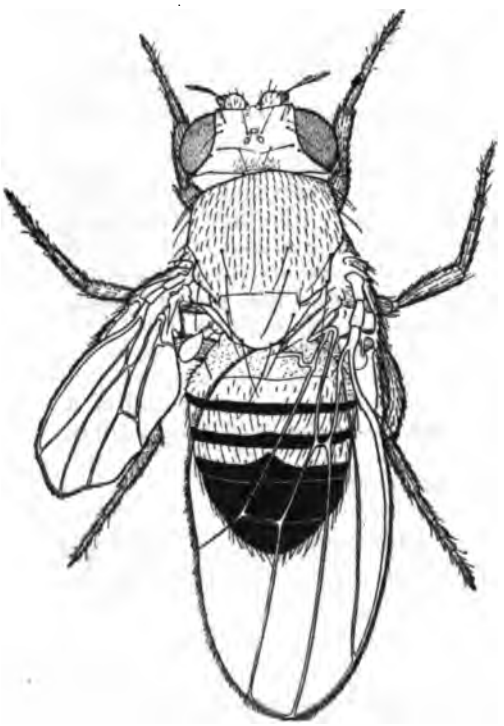
Explanations.—An egg containing a cross-over chromosome with the genes for notch, tan, and vermilion was fertilized by an X sperm carrying eosin, tan, and vermilion. Elimination of the maternal X was followed by shifting of the cleavage nuclei.



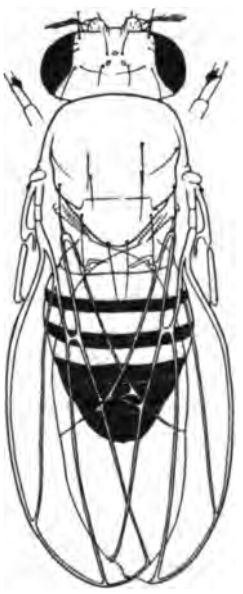
No. 983. December 20, 1914. C. B. Bridges. Text-figure 47 (diagram).

Parentage.—One of the X chromosomes of the mother carried the genes for white and for bar, and the other X the gene for eosin. The father was miniature.

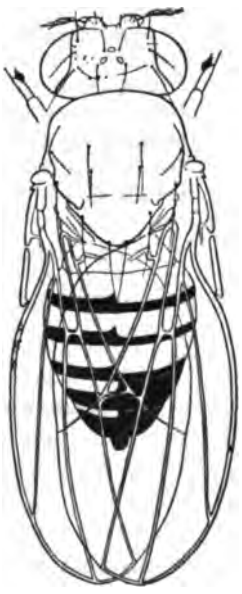
Description.—The separation of the sex-characters is very complex. The dorsal parts of the thorax and the wings are, from their size, female; the lower



TEXT-FIGURE 45.



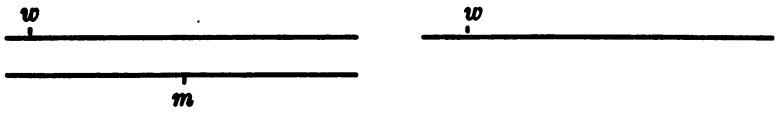
TEXT-FIGURE 46.



TEXT-FIGURE 47.

part of the thorax, from the presence of sex-combs on both forelegs, is male. The abdomen is female on the left half and male on the right. The genitalia are female. The abdomen contained a pair of ovaries as seen through the body-wall and in sections. The fly was sterile. The head was entirely male, with white eyes, not-bar.

Explanations.—A cross-over X carrying the gene for white but not for bar was present in the egg, which was fertilized by the X sperm carrying the gene for miniature. Elimination of this paternal X left a cell with the white X to determine the male parts. In the early cleavage there must have been extensive shifting of the nuclei to produce the observed mosaic of female and male parts.

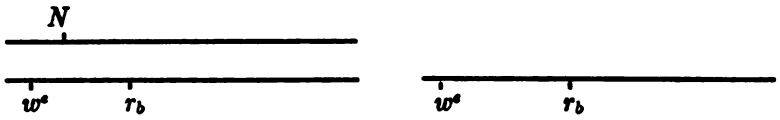


No. 16240521114. Selection Experiment. August 2, 1916. T. H. Morgan. Plate 4, Figure 2 (drawing).

Parentage.—The gynandromorph arose in a "selected" notch stock in which the female carried notch in one X and eosin and ruby in the other. The father was eosin ruby.

Description.—The gynandromorph was "quartered," being male in the anterior left section and also in the posterior right section, and female in the two other sections. The left eye was mainly eosin ruby, but had a small section of red (female) pushed in from the rear. The left side of the thorax was male, as evidenced by the sex-comb and the shorter wing. The right side of the abdomen had male coloration above and below, and the genitalia were male on the right side and female on the left. The abdomen seemed to have a pair of ovaries when examined, but the sections made later were too poor to confirm this. The right eye was red and the right wing notch.

Explanations.—An egg carrying the gene for notch was fertilized by a sperm carrying the genes for eosin and ruby. Elimination of the maternal notch X occurred at the first division, leaving the eosin ruby paternal X to determine the character of the male parts. The products of the second division rearranged themselves so that sister cells took part in the development of opposite sides of the body. This is only a little more extreme than the usual rearrangement and shifting of parts (see patch of red in left eye).



SPECIAL CASES.

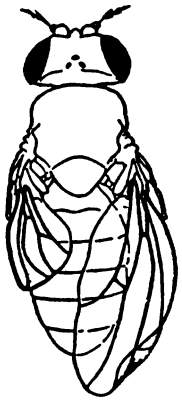
The following cases were brought together because they could not be explained simply by the theory of elimination. Analysis showed that in each of these cases there were present two different chromosomes, *both derived from the mother*. Non-disjunction obviously offered an explanation for this fact. But the application of this hypothesis required the additional assumption of "somatic reduction" to explain the gynandromorphism. This means that at an early division the two X's derived from the mother separate without division. On the other hand, if we assume for these cases that both sex chromosomes leave a daughter half at the mid-plate (double elimination) the assumption just stated is avoided. Until further explanation is obtained these two interpretations may be given as alternatives.

Doncaster's observations on binucleated eggs of *Abraxas*, where both nuclei underwent separate reduction and fertilization, offer a simpler explanation. On the other hand, it should be pointed out that there should have been at least as many autosomal mosaics as sex-linked mosaics produced by fertilization of binucleated eggs of heterozygous mothers; and this does not seem to be the case.

No. B. 90. June 17, 1912. C. B. Bridges. Text-figure 48 (drawing).

Parentage.—This gynandromorph appeared in F_2 from the cross of rudimentary female to white miniature male; that is, the mother (F_1 female) carried rudimentary in one X and white and miniature in the other; and the father was a rudimentary (F_1) male.

Description.—The individual seemed to be male throughout. Both eyes were red. Sex-combs were present on both forelegs. The right wing was long, and though slightly deformed, was undoubtedly wild-type. The left wing was a typical and perfect miniature rudimentary wing. The abdomen was entirely male, and when mated to a vermilion female the fly bred as a male, producing abundant offspring. Several pairs of the wild-type daughters and vermilion sons of this mating were bred and all produced red and vermilion in equal numbers, both in males and females. That is, the gynandromorph bred as a wild-type male carrying no mutant genes. Two of the F_2 pairs are given as samples:



TEXT-FIGURE 48.

	Wild-type ♀	Wild-type ♂	Vermilion ♀	Vermilion ♂
B. 98.1	45	33	32	43
B. 98.2	26	16	30	33

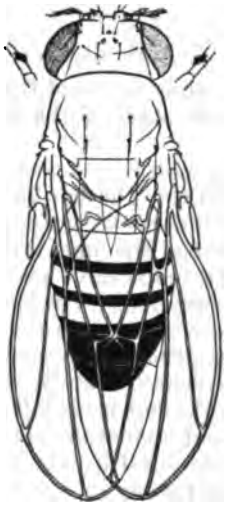
The drawing has been previously figured in Zeit. f. ind. Abst. und Verer., 1912, p. 324.

Explanations.—Simple elimination fails to explain this case, because the characters of the fly, as well as its genetic behavior, show that it received two different X chromosomes from its mother. For instance, miniature and rudimentary were both present in the left (male) wing, which proves that the X contained in these parts came from the mother and that crossing-over in the mother must have occurred. Since the right wing was wild-type, its cells must have contained a wild-type X, which likewise could only have come from the mother. The F_1 and F_2 offspring of the gynandromorph showed that he had such a wild-type X in the testis, which presumably came from the same kind of cells as those of the right side. The offspring also show that the gynandromorph had not received an X sperm from the father, which would have given rudimentary offspring. Therefore the right side, at least, must have come from a Y-bearing sperm, as further proved by the fact that the gynandromorph was fertile as a male (males without a Y being sterile).

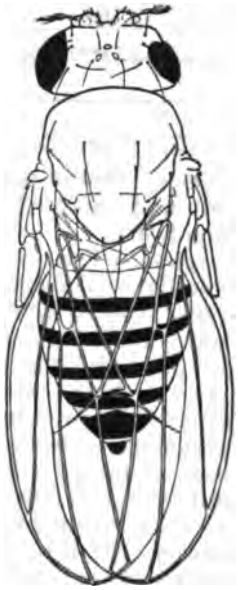
On the view that the gynandromorph came from an egg with two nuclei, a simple explanation of the result may be given. Before reduction, each of the postulated nuclei must have had one white miniature X and one red rudimentary X chromosome; after crossing-over and reduction in each, one

nucleus contained a white miniature rudimentary cross-over X, and the other nucleus a wild-type cross-over X. Each nucleus was fertilized by a Y-type sperm, proof of which for the right side has been given; proof for left side is as follows: The left wing is miniature as well as rudimentary, and since

TEXT-FIGURE 49.



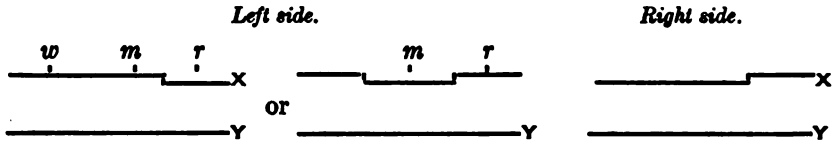
TEXT-FIGURE 50.



TEXT-FIGURE 51.

the X of the father did not carry miniature, this left side could not have contained a paternal X and must therefore have contained a paternal Y chromosome.

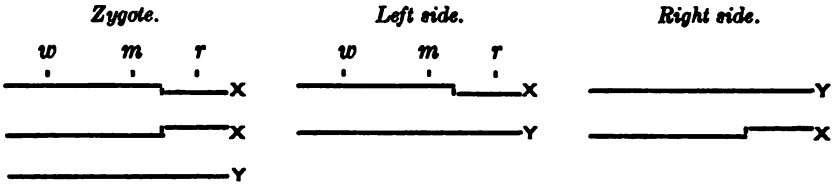
One of the cross-over chromosomes was white as well as miniature and rudimentary; but since the eye on the side with miniature was red, we may suppose that all of the head came, as is very often the case, from cells from one side only, namely, the right, which was here carrying red; or this cross-over chromosome may have come from double crossing-over, and in this case it would have carried red.



On an alternative view that both of these X's were in a single nucleus, the following assumption seems necessary. An XX egg was produced by reductional primary non-disjunction (see Bridges, 1916), preceded by crossing-over, so that one X contained white miniature rudimentary and the other was the complementary X containing only wild-type genes. This XX egg was then fertilized by a Y sperm.

That the individual was entirely male with no female parts can be explained either by double elimination or somatic reduction at the first division of the zygote; that is, one member of each pair was caught by the elimination plate,

so that each of the two first daughter cells had but one X and these different from each other.

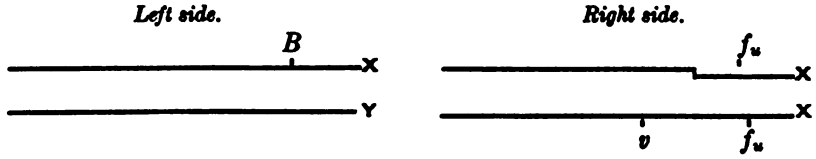


No. I 92. August 16, 1913. C. B. Bridges. Text-figure 49 (diagram).

Parentage.—One of the X chromosomes of the mother carried the genes for vermilion and for fused and the other X the gene for bar. The father was vermilion fused.

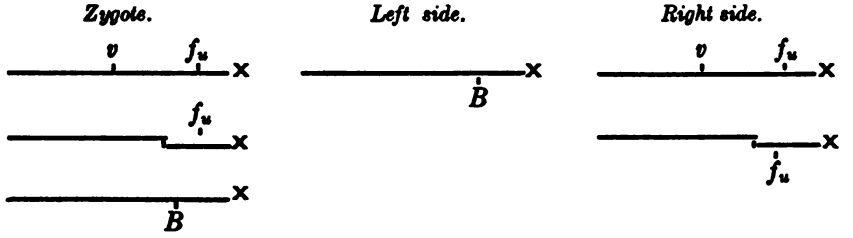
Description.—The gynandromorph was completely bilateral, except for the genitalia, which were female. The left side was male, as evidenced by the smaller size throughout, by the sex-comb, and by male coloration on the abdomen. The left eye was bar of the male type. The right side was female in every part, and was chiefly remarkable in that its large wing was fused. The eyes were both red, not vermilion. The right eye was round, not heterozygous bar. A pair of ovaries was found in the sections.

Explanations.—On the assumption of two nuclei in the egg, one nucleus after reduction contained a non-cross-over bar X chromosome, and this nucleus fertilized by a Y sperm gave the bar male left side, with bar eye; the other nucleus after crossing-over and reduction contained a cross-over fused X chromosome, which nucleus fertilized by the vermilion fused X sperm gave the female right side with fused wing:



On the alternative view that both X's from the mother were retained after reduction in the same nucleus of the egg, the case is difficult, but may be accounted for in the following way: Since the left side is male throughout and shows the bar eye-character (of male type), this side must have come from a non-cross-over X of the mother. But this bar X is not represented at all on the right side, as proved by the round eye, which, although female, is not even heterozygous for bar. That the right side is female requires that two X's be present, and the fact that the wing is fused requires that both carry the fused gene. A non-cross-over vermilion fused X must have come from the mother along with the bar X. The egg, then, was an XX egg produced by primary non-disjunction which was equational, since the bar X was a non-cross-over and the fused X a cross-over chromosome (Bridges, 1916). This XX egg was fertilized by an X sperm carrying the genes for vermilion and for fused. It is known that XXX zygotes are unable to hatch as adult flies (Bridges, 1916), but since neither the time nor the mechanism of their elimination is known, it is possible that if double elimination or somatic reduction followed soon after fertilization the life of the XXX individual would be saved, *but at the price of becoming a gynandromorph.* Two of the X's, in this case the paternal vermilion fused and the maternal

fused cross-over X, remained in one cleavage cell which gave rise to the not-vermilion not-bar fused female right side. The other X, the maternal non-cross-over bar X, passed into the other daughter cell and gave rise to the not-vermilion bar not-fused left side.

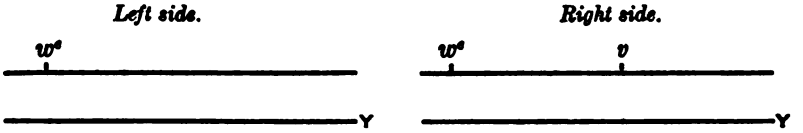


No. 937. December 17, 1914. C. B. Bridges. Text-figure 50 (diagram).

Parentage.—The grandmother was a wild-type XXY female carrying the genes for eosin and vermilion in one X and in the other only wild-type genes; the grandfather was white bar. By equational non-disjunction an XXY eosin daughter was produced which carried eosin and vermilion in one X and eosin in the other. This female was out-crossed to a vermilion male and produced among the sons a mosaic.

Description.—The mosaic, as in the case B 90, was male throughout, but the left eye was eosin (of the male type) and the right eye was eosin vermilion. The male was fertile when bred to a vermilion female, giving wild-type daughters and vermilion sons (No. 1116). One of the wild-type daughters out-crossed to a forked male gave eosin and vermilion as the main classes of the sons.

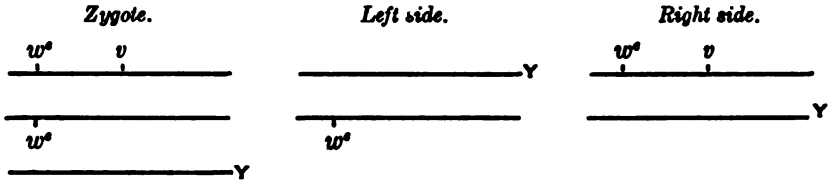
Explanations.—On the hypothesis of a binucleated egg, one nucleus after reduction contained an eosin vermilion X and the other nucleus an eosin X. Since no eye-color corresponded to the X sperm of the father, and since the individual was male throughout, both of the egg-nuclei must have been fertilized by a Y sperm, which is further shown by the fertility of the male.



On the view that a single nucleus was present, the following situation develops: Since the right eye showed both eosin and vermilion, the mosaic must have contained the eosin vermilion X of the mother. Since the other eye showed eosin (not vermilion), this X must have been the other or eosin X of the mother. That is, both X chromosomes of the mosaic came from the mother by means of an XX egg produced through non-disjunction. The vermilion X of the father was not present at all, as proved by the fact that the left eye of the mosaic was eosin (not red) and male (not female), and by the breeding-test, which showed that the gonads carried only the eosin X. The sperm was not the X sperm of the father, but the Y sperm, as further indicated by the fertility of the male.

As in case B 90, there must have been double elimination or somatic reduction, so that one cleavage-cell received the eosin X and a Y, and the other

the eosin vermilion X and a Y. The gonads developed from an eosin cell as shown by the F_1 and F_2 results of his breeding test.

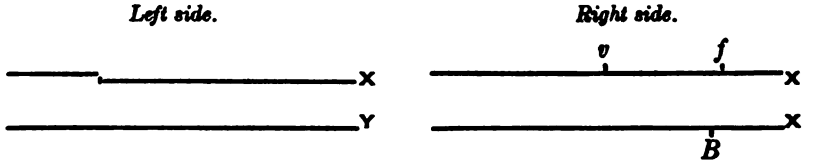


No. 1333. February 19, 1915. C. B. Bridges. Text-figure 51 (diagram).

Parentage.—The mother was a wild-type XXY female, carrying the genes for eosin in one X and for vermilion and forked in the other. The father was bar.

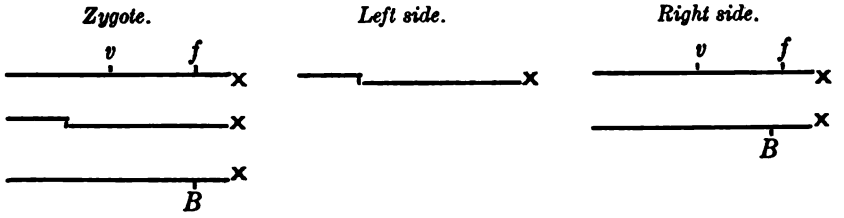
Description.—The fly was female throughout, except for the left eye which was round (not bar) and red (not eosin or vermilion). The eye has been examined repeatedly at different times since the mosaic was on hand, and the eye is undoubtedly not-bar and is of the right size for a male. The right eye was heterozygous bar. There were no forked bristles present around the left eye elsewhere. The female was mated to a sable forked male and produced: No. 1555—forked females, 11; forked bar females, 0; bar females, 18; wild-type female, 1; vermilion forked males, 18; bar males, 6; vermilion bar males, 3; forked males, 2.

Explanations.—On the hypothesis of a binucleated egg, one nucleus after reduction contained a cross-over wild-type X and the other a non-cross-over vermilion forked X chromosome. The former fertilized by a Y sperm gave rise to the wild-type (male) left eye; the latter fertilized by a bar X sperm gave rise to the rest of the fly.



The following alternative possibilities may be considered: The simplest possible explanation is that this is a mosaic or somatic mutation—that the bar gene in the cell that gave rise to the left eye reverted to not-bar, or to an allelomorph which gives a small round eye. If, as is more probable, this mosaic is a gynandromorph arising by chromosomal disturbance, the explanation is like that for No. I 92, *i. e.*, the egg arose by equational non-disjunction and contained a non-cross-over vermilion forked X and a cross-over wild-type X. This egg probably did not contain a Y, as evidenced by the lack of exceptions among the sons of the mosaic, and as is possible in accordance with the assumption of equational non-disjunction, for equational non-disjunction, even when occurring in a female with a Y, is probably always primary. One eye was clearly heterozygous bar; hence it is known that the XX egg was fertilized by an X sperm carrying the gene for bar. This XXX zygote would ultimately die, unless at an early stage the XXX condition was corrected by reduction or elimination. Double elimination or somatic reduction in a cleavage-cell would save the individual, but turn it into a gynandromorph. The other X chromosome, wild-type, passed into the sister cell and gave rise

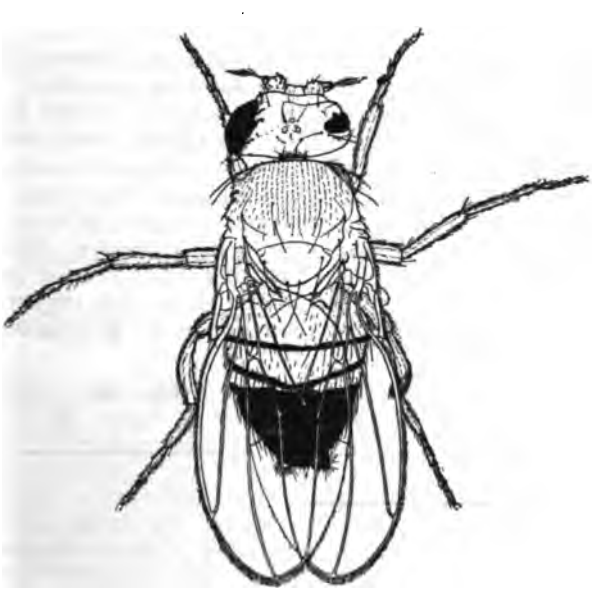
to male parts, which, because of the lateness of the occurrence, or from shifting of nuclei, constituted but a small part of the gynandromorph.



No. 2349. November 3, 1915. C. B. Bridges. Text-figure 52 (drawing).

Parentage.—The mother was from a strain of high non-disjunction, but was known to be XX and not XXY. One X carried the genes for vermilion and forked, the other X the gene for bar. The father was a vermilion forked male.

Description.—The gynandromorph was largely male. The female parts included the left legs, which were without a sex-comb and had forked bristles. The female parts throughout had forked bristles and could therefore be readily traced. All three left legs were forked and female to the mid-ventral line. A very narrow strip of female tissue ran diagonally forward from above



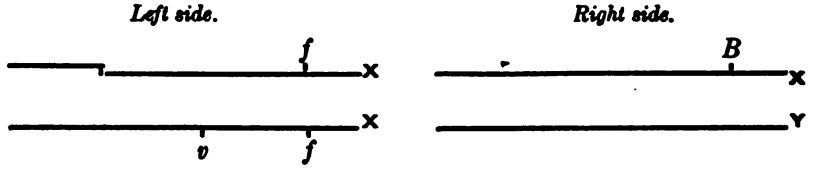
TEXT-FIGURE 52.

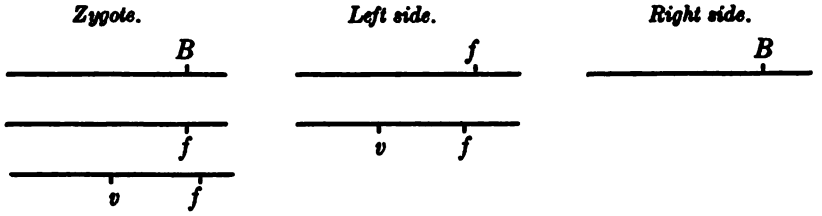
TEXT-FIGURE 53.

the middle left leg to the shoulder, being chiefly marked by one large forked bristle and several smaller ones. Most of the left side of the head bore forked bristles, including the left antenna, the dorsal region to the left of the line in the diagram, a small zone of tissue around the eye to the rear, and the region below the eye including the oral bristles. The left eye was red (not vermilion) and round (not bar or heterozygous bar—the small nick seen in the drawing of the eye seems to be an artifact). The abdomen was male type, the genitalia

were half and half, the left half bearing a female type anal prominence with forked bristles. Sections showed that ovaries were present with well-developed eggs, which account for the large size anteriorly of male-type abdomen. The male parts, as distinguished by the normal bristles, included the whole dorsal surface of the thorax, the two wings, which were of equal size (male), the abdomen (except for half the genitalia), all the right legs, and somewhat more than the right side of the head. The right foreleg bore a sex-comb. The right eye was bar (male type), not vermilion in color.

Explanations.—On the theory that two nuclei were present in the egg, one nucleus contained after reduction a cross-over forked X, the other nucleus a bar X chromosome. The former fertilized by a vermilion forked X sperm gave rise to the female parts on the left side, the latter fertilized by a Y sperm gave rise to the left male side, with the bar eye, etc.



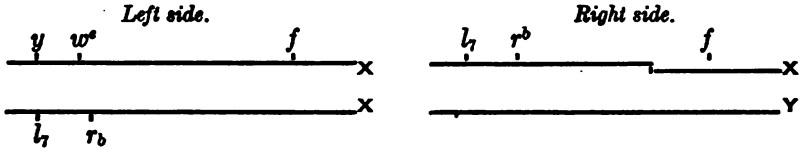
On the assumption of a single nucleus in the egg a possible explanation is as follows: The male parts show the character bar, and since bar was present only in the mother, they are known to have been derived from the maternal bar X, which was a non-cross-over X, since the eye did not show the character vermilion. The female parts were forked, but since the eye was not vermilion, one of the forked X's must have been a cross-over between vermilion and forked. Crossing-over takes place only in the female and not in the male, wherefore this X also is known to have come from the mother. One cross-over and one non-cross-over X is the general rule for eggs produced by primary equational non-disjunction. The other forked X must have come from the father and therefore carried the gene for vermilion; but vermilion is recessive and its effect is hidden by the normal allelomorph in the cross-over X from the mother. The gynandromorph, as in cases 192 and 1333, started as a XXX zygote which was saved from death and at the same time converted into a mosaic by double elimination or somatic reduction at the first cleavage division.

No. 4241. May 15, 1916. C. B. Bridges. Text-figure 53 (diagram).

Parentage.—One of the X chromosomes of the mother carried the genes for lethal 7 and ruby eye-color, the other X the genes for yellow, eosin, and forked. The X chromosome of the father carried the genes for yellow, eosin, and forked.

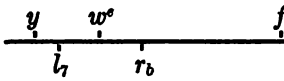
Description.—The right side was male throughout, except that in the head the female part (bordered by the dashed line in the diagram) extended nearly to but did not include the right eye. The right eye was smaller and ruby.

The two anterior bristles above the right eye and all the bristles below it were forked, agreeing with the forked bristles present throughout the rest of the right side on legs, thorax, and abdomen. A sex-comb was present on the right fore-leg and all male parts were smaller. The right side was female throughout, with normal bristles and a red eye. The genitalia were half-and-half. In the sections of the abdomen an ovary could be identified on one side, less certainly on the other. The body-color of both male and female parts was wild-type throughout, with no yellow.

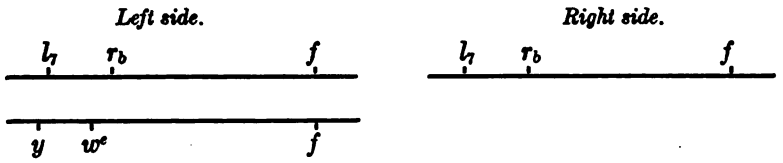
Explanations.—On the theory of the binucleated egg, one nucleus contained an X with the genes for lethal and ruby, the other nucleus a cross-over X with the genes for lethal, ruby, and forked. The former fertilized by X sperm with yellow eosin forked, produced a female left side with only wild-type characters; the latter, fertilized by a Y sperm, gave the ruby forked male side.



An alternative view based on a single nucleus is as follows: Simple elimination fails to explain the case, since the male parts that are forked are not-yellow and not-eosin, as might be expected, but instead were ruby. Since ruby was present only in the mother, the male parts must have come from a lethal 7 ruby forked cross-over chromosome produced by the mother. That the other X chromosome of the zygote was not the yellow eosin forked X of the father is proved by the not-forked character of the female parts. It seems certain that both X chromosomes of the zygote came from the mother, that is, that the egg was a non-disjunctional XX egg. This must have been by primary non-disjunction, since the pedigree is fully known and no other exceptions were produced. Another fact points to the same conclusion, namely, that these X's were both cross-overs, and, as Bridges has shown, both X's of secondary exceptions are always non-cross-overs. What occurred, then, was crossing-over in the



female between the loci ruby and forked. Owing, perhaps, to some entanglement in the process of crossing-over, the chromosomes were unable to separate in time for the reduction division and both were retained in the egg. This egg, containing a yellow eosin X and a lethal 7 ruby forked X, was fertilized by a Y sperm. At the first segmentation divisions one of these maternal yellow eosin chromosomes was eliminated, giving a gynandromorph whose male parts were lethal 7, ruby, and forked.



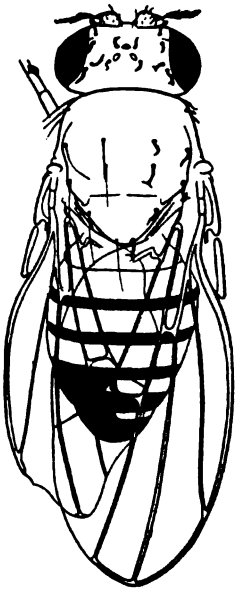
A very interesting point in connection with gynandromorph 4241 is the fact that a male part, which must be assumed to have the lethal 7 gene, was able to live when associated with the not-lethal partner in the gynandromorph. This is, however, understandable when the nature of the action of the lethal 7

gene is considered. Normally, the males which possess the lethal 7 gene not only begin development, but continue often to the full-grown larva stage. The immediate cause of their death at this late stage is the development of one or more black granules which cause death either because they are themselves toxic or because their substance is derived from an excessive malformation of organs essential to the further development of the fly. On the first view, either the half amount of toxic body was insufficient to prevent the gynandromorph from continuing its development, or the corresponding normal parts of the female side counteracted this toxic effect; on the second view, the lack of the essential organ on the male side was supplied by the normal organ of the female side.

No. 3674. August 9, 1917. A. H. Sturtevant. Text-figure 54 (diagram).

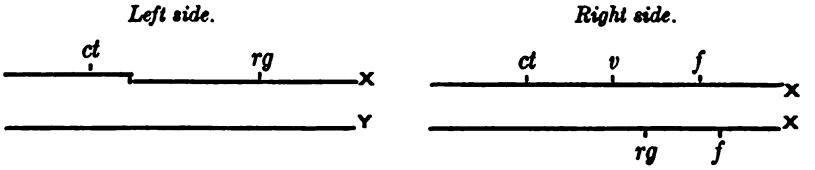
Parentage.—One of the X chromosomes of the mother carried the genes for cut, vermilion, and for forked; the other X the gene for rugose. The father was rugose forked.

Description.—The male parts of the gynandromorph constituted the left side of the thorax and abdomen, as indicated by the smaller size, the sex-comb, the smaller wing, and the male coloration of that side of the abdomen. Testes were found in the abdomen. The left wing showed the character cut. The bristles of all male parts were wild-type. The bristles of the female parts were forked, and this character forms the most useful index of the division-line. The entire head, including the bristles above and below and around the left as well as the right eye, was forked. The first and second leg on the right side were forked, the third was not forked and presumably therefore male. The bristles on the right wing and on the right side of the abdomen were forked. There was no sex-comb on the right side. The right wing was of female size and not cut. Both eyes were red (not vermilion) and also not rugose.



TEXT-FIGURE 54.

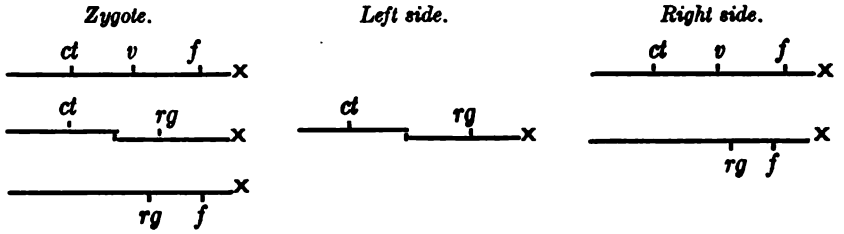
Explanations.—On the theory of two nuclei, one nucleus contained a cross-over X with the gene for cut and rugose, the other an X with the genes for cut vermilion forked. The former nucleus was fertilized by a Y sperm to produce the left side, the latter nucleus by an X sperm with rugose and forked to produce the female right side.



An alternative explanation on the assumption of a single nucleus follows: The mechanism in this case must be essentially the same as in cases I 92, 1333, and 2349 already given. The female parts were entirely forked, therefore two forked chromosomes were present. One of these could have come from the father, whose X was rugose forked; the other X could have come from

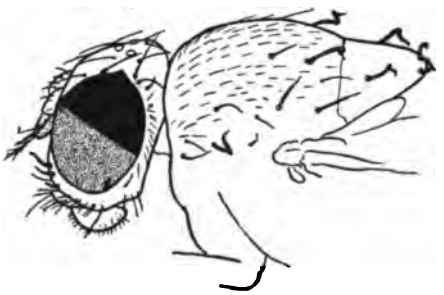
the mother, and could have been the non-cross-over cut vermilion forked X. Neither cut nor vermilion would show in the female parts, since they would be recessive to their wild-type allelomorphs in the rugose forked X; and likewise rugose would not show, for it would be recessive to its wild-type allelomorph in the cut vermilion forked X. Both of these X's could not have come from the father, for in that case both eyes would have been rugose. One of the forked X's therefore came from the mother. The left wing was cut, and since cut was present only in the mother, this X also must have come from the mother. Since the cut side did not show forked, this cut X must have been a cross-over anywhere between cut and forked. Thus we see that the egg contained two X's which were different, one being the non-cross-over cut vermilion forked X and the other the cross-over cut X, which is the normal condition of XX eggs produced by primary equational non-disjunction. This XX egg was fertilized by the rugose forked X sperm of the father, giving an XXX zygote. At the first segmentation division, double elimination or somatic reduction occurred, thereby enabling the fly to survive, but only at the price of becoming a gynandromorph. The paternal (rugose forked) and one of the maternal X's (cut vermilion forked) entered one cell, from which developed the female right side, which showed only one mutant character, namely, forked. The cross-over maternal X (cut vermilion? rugose? not-forked) entered the other cell and gave rise to the male left side, showing the mutant character cut only.

It should be noticed that in all four of these cases it has been the paternal X and one of the maternal X's that have come together into the female part, and that the male part was in each case maternal. This suggests that the essential feature of the reduction is the active separation of the two X's which abnormally came from the same individual and the passive inclusion of the paternal X in the same cell with either separated maternal X.



No. 7730. October 24, 1917. C. B. Bridges. Text-figure 55 (drawing).

Parentage.—The mother was a wild-type regular XX female (from a strain of high non-disjunction) carrying the genes for eosin and forked in one X and only wild-type genes in the other. The father was bar. No exceptions were produced other than the following gynandromorph.



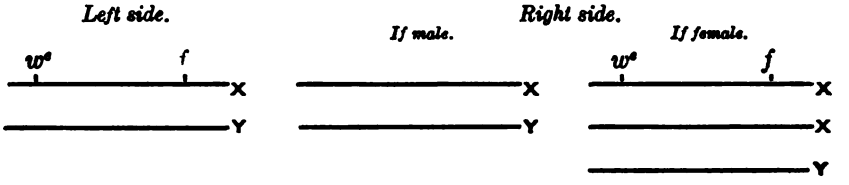
TEXT-FIGURE 55.

Description.—The gynandromorph was almost entirely male. All parts, except the head, were male and had forked bristles. The head was mainly female, having straight bristles and a red (not-bar) eye on the right side, and on the left side a division-line which ran forward through the eye. Above this line, which was perfectly clean and

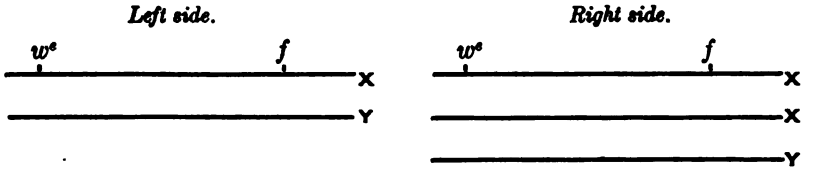
sharp, the eye was red and the bristles wild-type; below the line the eye-color was eosin (male type) and the bristles were clearly forked. It is possible that the not-eosin, not-forked part, described above as female, was really male, in which case the fly would be a male mosaic. The fly, bred as a male to an eosin-crimson female, produced 143 wild-type daughters and 151 eosin crimson sons. Two pairs of these were inbred and produced:

No.	Eosin ♀	Eosin crimson ♀	Eosin crimson ♂	Eosin forked ♂	Eosin ♂	Eosin crimson forked ♂
8056	68	83	41	44	26	32
8057	75	84	45	46	24	25

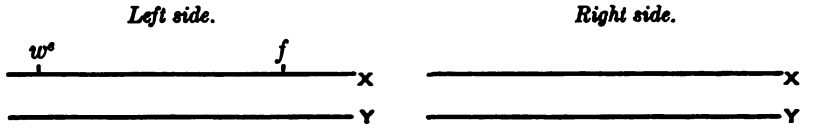
Explanations.—On the assumption that two nuclei were in the egg and that the fly was entirely male, one nucleus contained an eosin forked X and the other nucleus a wild-type X; each nucleus having been fertilized by a Y sperm, the former gave rise to the eosin forked male parts and the latter to wild-type male parts. In case the red parts were female the corresponding nuclei must have come from an XX egg produced by primary non-disjunction, and likewise fertilized by a Y sperm.



On the view that only one nucleus was present in the egg, the possible explanation is as follows: On the assumption that the wild-type parts were female, both of the X's present must have come from the mother, since the eye was not-bar, as would have been the case if the X of the father were present. Moreover, that the sperm was the Y sperm is known from the fact that the mother had no Y to contribute and yet the fly was fertile. The egg was therefore XX, one X being eosin forked and the other wild-type, and was produced by direct primary non-disjunction. Elimination of the wild-type X occurred and the male cell gave rise to most of the body, including the gonads.



On the assumption that the wild-type parts were male, the zygote must have had the same origin as above, but double elimination (or somatic reduction) occurred, so that one cell received a single eosin forked X and the other a single wild-type X.

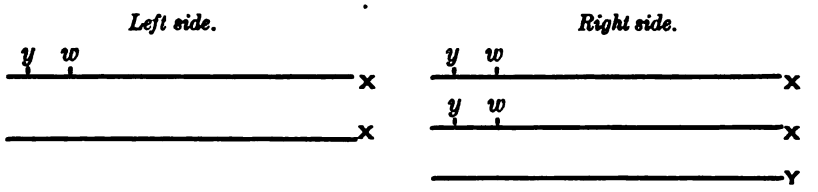


No. G_1a, b_2, c. April 1914. E. M. Wallace. Plate 3, figure 4 (colored drawing).

Parentage.—The mother was a white-eosin compound female, carrying the genes for yellow and white in one X and eosin in the other. The father was an ebony male, used to show the lack of elimination of the third chromosome.

Description.—The mosaic was entirely female. The right side of the thorax, the right wing, and the right legs were yellow in color, while the rest of the female, including all of the head and abdomen, was gray. Both wings were of the same size and there was no size inequality in bristles or other parts. There was no sex-comb on the yellow right foreleg.

Explanations.—On the view that this gynandromorph arose from a binucleated egg, it must be assumed that one of these nuclei must have contained two yellow white bearing X's that arose through equational non-disjunction; the other nucleus contained (as the offspring showed) a yellow white chromosome. The former nucleus fertilized by a Y sperm gave the yellow parts of the fly (not including right side of head, which is gray red); the latter nucleus fertilized by wild-type X sperm (from the ebony male) gave the left side of the fly, including all of head and abdomen.



If the mosaic had arisen from a yellow white egg fertilized by the X sperm of the ebony male (whose X chromosome carried only wild-type genes) it would have been easy to explain the case as simple elimination were it not that the yellow parts were unmistakably *female*, which is impossible without the additional hypothesis of a succeeding somatic non-disjunction. It was next supposed that the mechanism of the production of the mosaic had been double somatic non-disjunction, that the two daughter wild-type X's had gone into the same cell, giving rise to the wild-type left-side female parts, and that the two daughter yellow white X's had both been included in the other cell, which gave rise to the yellow female parts on the right side. On this hypothesis the offspring (disregarding ebony) should correspond to those of a pure yellow white female or of a pure wild female. In fact, however, the offspring correspond to those of the original zygote when the mosaic was mated to a yellow white brother: yellow white females, 106; yellow white males, 103; wild-type females, 117; wild-type males, 107; yellow males, 2; white male, 1.

A possible escape from this dilemma is to suppose that the non-disjunction took place after the first division and that the normal cell was the one which gave rise to the germ-cells. This mosaic would then be triregional—the abdomen and gonads heterozygous for yellow and white and representing the original zygote, the right side of the thorax pure yellow white, the left side of the thorax and the head pure wild-type.

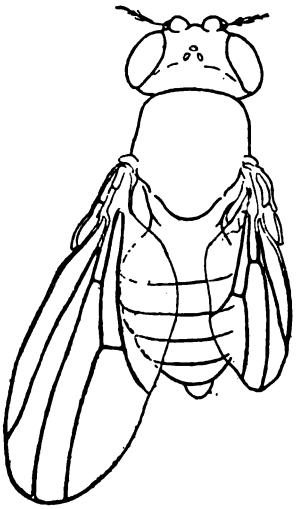
Another type of explanation is that in the normal XX zygote somatic mutation to yellow occurred in the wild-type chromosome, so that the yellow part contains a mutant yellow X and the maternal yellow white X. Or somatic deficiency for the yellow locus occurred in the wild-type X, so that the yellow parts are haploid for yellow, and like the normally haploid male show yellow, while these parts are female because the sex gene is situated in some other part of the X than the yellow-deficient region.

GYNANDROMORPHS WITH INCOMPLETE DATA.

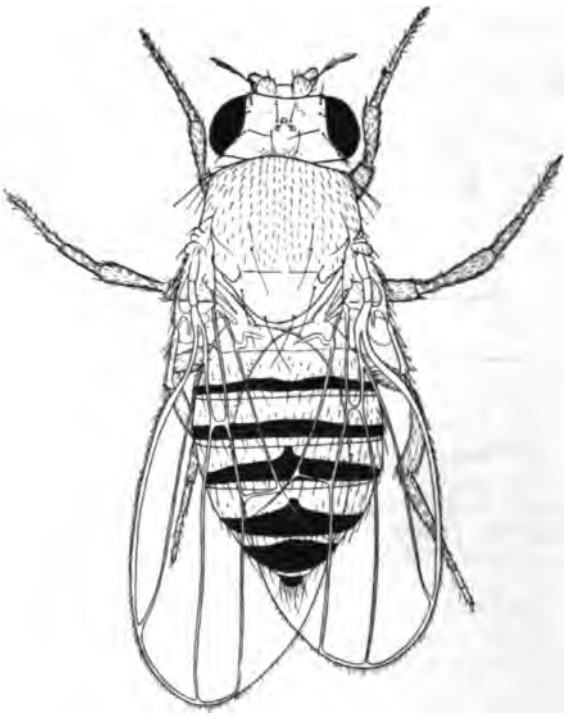
A fly was figured by Morgan (Zeit. f. i. Abst. u. Ver. VII 1912, fig. 3) with one long wing and one miniature wing (text-fig. 56). Its history has been lost, but it is recorded in a paper giving crosses that involve miniature wings. The fly was probably a gynandromorph.

No. 28. February 11, 1918. T. H. Morgan. Text-figure 57 (drawing).

Parentage.—Uncertain; probably the gynandromorph appeared in a stock of "serrate" extracted from a cross of dichæte (carrying serrate) to short notch.



TEXT-FIGURE 56.



TEXT-FIGURE 57.

Description.—The gynandromorph was largely female, the entire head and the right side of the thorax with the right wing and legs being male. The sex-combs seemed to be only half as large as that of a normal male.

No. M. February 1912. E. M. Wallace. Text-figure 58 (diagram).

Parentage.—The ancestry is unknown.

Description.—The gynandromorph was largely female; the male parts being the right dorsal half of the thorax with its wing, which were yellow in color and of smaller size.

No. H. July 1913. Text-figure 59 (diagram).

Parentage.—Ancestry unknown.

Description.—The fly was yellow and female to all appearances, except tip of abdomen, which was male. A penis was present. Sections showed one abnormal testis and one broken one.

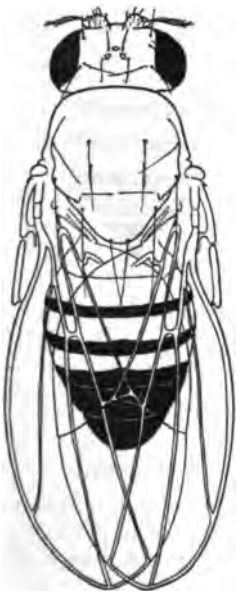
No. G. May 1914. T. H. Morgan. Text-figure 60 (diagram).

The ancestry is unknown. The gynandromorph was mainly female. The fly was gray with red eyes. There were no sex-combs and the wings were equal in length. The tip of the abdomen had male banding. A pair of ovaries were seen through the body-wall and eggs were found in section.

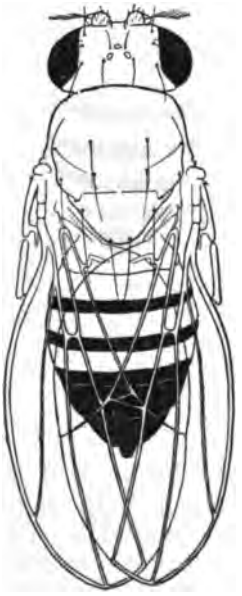
No. N. September 1916. T. H. Morgan. Text-figure 61 (diagram).

Parentage.—The ancestry is uncertain; probably the gynandromorph came from the notch stock. If so, the mother carried notch in one X and eosin in the other, and the father was eosin.

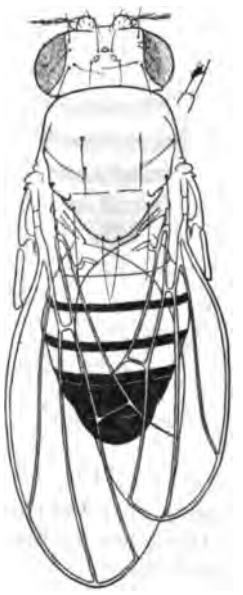
Description.—The gynandromorph was largely male. The entire abdomen, the right half of thorax, with wing and legs, were male. The division



TEXT-FIGURE 58.



TEXT-FIGURE 59.



TEXT-FIGURE 60.

between male and female in the head ran through the right eye, which was light eosin (male) below and dark eosin (female) on the dorsal half.

No. O. January 1912. Text-figure 62.

The ancestry not recorded. It had one red eye (right) and one white eye with a red fleck in it (left). On the left side there was a sex-comb. The wings were equal in length and apparently female. The abdomen and genitalia were entirely female. Poorly developed eggs were seen in section.

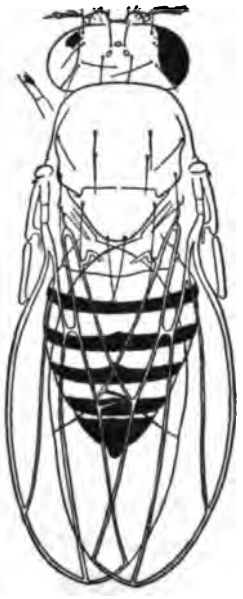
No. P. December 1913. Text-figure 63.

The ancestry of this gynandromorph is not recorded. It has a sex-comb and short wing on the right side. The abdomen was mostly female, but showed some male parts in the genitalia.

No. X. July 15, 1916. Text-figure 64.

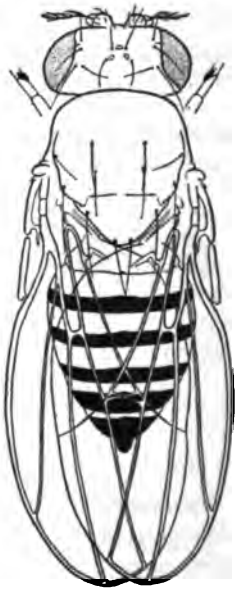
Parentage.—Ancestry unknown.

Description.—The head was small and apparently therefore male. The eyes were eosin ruby. Sex-combs were present on both sides. The abdomen was female. This is apparently an antero-posterior mosaic.



TEXT-FIGURE 61.

TEXT-FIGURE 62.



TEXT-FIGURE 63.

No. 110. December 12, 1915. A. Weinstein. No diagram.

The mother had the genes for eosin, ruby, and forked in one X chromosome. and the genes for fused in the other. The father was bar. The eyes of the gynandromorph were bar (homozygous or heterozygous?); the wings were abnormal; the abdomen was female, with female genitalia somewhat abnormal.

DROSOPHILA GYNANDROMORPHS PREVIOUSLY PUBLISHED.

There are several references to cases where white spots were found in the eyes of *Drosophila*, sometimes in cases where the gene for white eyes might have been present (Amer. Naturalist, XLVXII, Aug. 1913, p. 509; Morgan, Science, XXXIII, Apr. 1911, p. 534).

No. I. J. S. Dexter, 1912. Biol. Bull. August 1912.

Parentage.—The mother carried the gene for yellow and white in one X and only wild-type genes in the other.

Description.—Although the individual is described as a female, it is more likely that the yellow white right side was male and the wild-type left side female. This female was found to be sterile, which agrees better with the assumption that the right side was male, since mosaics which are entirely or even more than half female usually are fertile.

Explanations.—A yellow white X egg was fertilized by a wild-type X sperm. Elimination of the paternal X occurred.

In the early stages of non-disjunction, C. B. Bridges (Journ. Exp. Zool, Nov. 1913) found several gynandromorphs, two of which (N_2 and (N_3) have been figured in *Heredity and Sex* (p. 163) and refigured here. Breeding-tests were tried on all these and it was shown that some were fertile, and further that the gynandromorphs were not due to an inherited condition. It was pointed out (p. 600) that such mosaic forms can be explained as due to somatic non-disjunction and also even to XXX zygotes.

Again, in the experiments on "Dilution Effects on Certain Eye Colors" (Morgan and Bridges, J. E. Z., Nov. 1913), about a dozen gynandromorphs were recorded, most of which were sterile, but those which bred (as females) behaved genetically as did their regular sisters; that is, they showed no trace in their gonads of the effect of the bodily division. This was especially striking in one case where the head was entirely white, yet in which the offspring showed eosin (pp. 44, 51).

No. I. F. N. Duncan. (See Am. Nat., vol. 49, p. 455, 1915.)

Parentage.—The father had white eyes, the mother was wild-type.

Description.—The fly had on one side a red eye, long wing, no sex-comb, and female abdomen. On the other side white eye, short wing, sex-comb, and male abdomen. Courted by males but would not court. Two testes with ripe sperm.

Explanations.—Elimination of a maternal X chromosome explains the results.

No. II. F. N. Duncan. Plate 3, figure 5 (colored drawing).

Parentage.—The male grandparent was cherry club vermilion, the female wild-type. The mother was heterozygous for the above genes. The father was wild-type.

Description.—The fly had a cherry left eye and red right eye. Sex-comb on left foreleg only. Right wing shorter than left. Abdomen largely female, more female left, more male right. Contained two testes with immature sperm.

Explanations.—An egg containing a cross-over cherry X was fertilized by an X sperm. Elimination of a paternal chromosome followed by an irregular distribution of the nuclei with one sex chromosome explains the results.

No. III. F. N. Duncan.

Parentage.—Same origin as No. II.

Description.—The fly had red eyes and sex-combs, left wing longer, abdomen male. Genitalia half male, half female. Was courted but would not mate. Two ovaries with ripe eggs.

Explanations.—Elimination of either a maternal or a paternal X chromosome will explain the result.

No. IV. F. N. Duncan.

Parentage.—Same origin as No. II.

Description.—Both eyes red, no sex-combs, wings same length. Abdomen and genitalia male on one side, female on other. Was courted. Two testes with mature sperm.

Explanations.—It is not possible to determine which X chromosome was eliminated.

No. I. 1915. Hyde and Powell. Genetics, I, 1916, p. 580 (colored diagram).

Parentage.—The mother was pure for blood, an allelomorph of white. The father was eosin, another allelomorph of white.

Description.—The mosaic was female except for the head, which was entirely male. The left eye was eosin (male type) and the right blood (*i. e.*, it was not eosin-blood compound).

Explanations.—A blood X egg was fertilized by an eosin X sperm. If at some cell division in the future head region of the very early embryo somatic reduction occurred, that is, if the eosin X went into one cell and the blood into the other, neither dividing, both cells would produce male parts with the eosin and blood type eyes. The result may, however, be explained in another way, viz, both chromosomes divided, but in an early cell division double elimination occurred. One daughter chromosome from each X was caught by the elimination plate, and the remaining X's were left, one in each cell.

No. II. 1916. Hyde and Powell.

Parentage.—The mother had white eyes and wild-type wings; the father had red eyes and truncate wings (second chromosome).

Description.—The gynandromorph had a white eye and a truncate wing on the left side and a red eye and wild-type wing on the right side. The fly was female in other parts and when mated to a white-eyed brother produced: red females, 75; white females, 70; red males, 65; white males, 65.

Explanations.—An egg containing a white-bearing X was fertilized by a red X sperm. Elimination of a maternal X left the male parts with the white X. The appearance and disappearance of truncate are so erratic that in this case no safe conclusion can be drawn from the appearance in only the male side. One might suppose that the male and female sides, differing in their X chromosomes, also differ in a sex-linked modifier for truncate.

GYNANDROMORPHS AND MOSAICS IN BEES.[1]

The domesticated bees have furnished many cases of gynandromorphs, both in hives supposedly pure and in hybrid communities. An excellent review of the recorded cases is given in Miss Mehling's paper of 1915. The earliest description is said to be that of Laubender in 1801. Lefebure in 1835, Donhoff in 1861, Smith in 1862, and Menzel in 1862 described gynandromorph bees. Widespread interest in the subject was aroused by the discovery of many gynandromorphs in the stock of an apiarist, Herr Eugster, in Constance. Menzel first reported on this occurrence. It was, however, von Siebold's account of the Eugster gynandromorphs (1868) that brought the subject to the general attention of zoologists. He gave not only a description of many of these bees, but dissected them also, and determined the correspondence or lack of correspondence between the internal sexual organs and the external sex characters. In this hive there was a queen of the yellow Italian race of bees (*Apis ligustica*) fertilized by a drone of the darker German race (*Apis mellifica*). Her sons were Italian, which is the expectation for this combination. After the death of the queen, another queen of "dark color" was present in the stock. She also produced some gynandromorphs.

[1]78 species of Hymenoptera in which gynandromorphs have been described are listed by Enderlein, including Tenthredinidæ, Braconidæ, Proctotrupidæ, Ichneumonidæ, Formicidæ, Mutillidæ, Crabonidæ, Scoliidæ, Pompilidæ, Vespidæ, and Aphidæ (11 families in all).

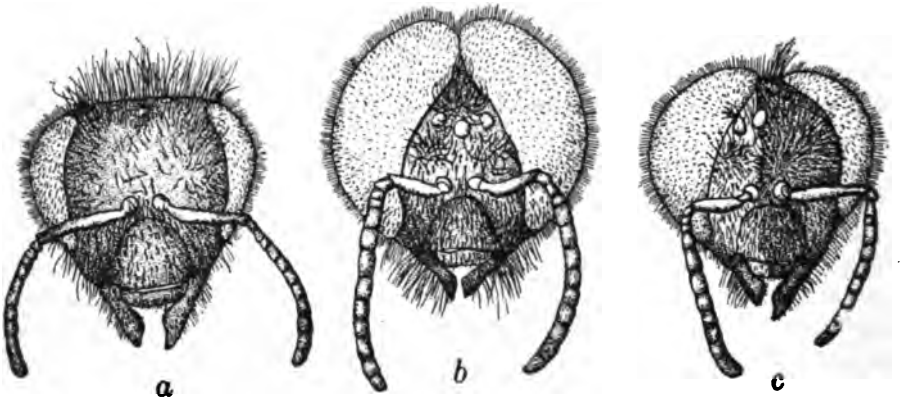
Several other descriptions of gynandromorphs in bees have been published (see Mehling, p. 174, and Dalla Torre and Friese, 1898).

There are certain facts in connection with sex determination in the bee that are almost unique and give an unusual interest to the situation. The queen has the double set of chromosomes which is reduced to the single or haploid number in the ripe egg, after two polar bodies are extruded. If the egg is fertilized it gives rise to a female (queen or worker), but if the egg is unfertilized it produces a male (drone). The male has only the single set of chromosomes. One set of chromosomes, then, produces a male, two a female; but whether sex is the result of special genes carried by one or two sex chromosomes has not been determined. Corresponding with the single (haploid) number of chromosomes in the male, the spermatogenesis shows certain special features. The preparation for the first division takes place, and only a small non-nucleated piece of protoplasm is pinched off at one pole of the cytoplasmic spindle. Preparation for a second division follows and the chromosomes separate into two groups, but the cytoplasmic division is very unequal and only one of the nucleated cells that results becomes a functional spermatozoon. That at the second division an equational division of the chromosomes occurs is probable, for in the closely related wasps the second division takes place normally (according to Mark and Copeland) and two spermatozoa are formed, each with the single number of chromosomes. Since in the male the haploid number of chromosomes must be supposed to be present, it might have been anticipated that his nuclei would be half the size of those in the corresponding parts of the female, as happens in the sea-urchin egg when haploid and diploid nuclei occur in different regions of the same embryo. An examination of this relation by Miss M. Oehninger has shown, however, that no such difference is present; hence what might have been a means of determining the constitution of the male and female parts of the gynandromorph is lacking.

Von Siebold found the male and female characters combined in many different ways in his gynandromorph bees, much as we find them in *Drosophila*. In some cases one side was male, the other female; or the anterior end might be like that of one sex and the posterior like that of the other; sometimes different regions of the same organ, such as an eye, leg, or antenna, might contain both male and female regions. The normal worker has a sting, the male is without this organ. In the gynandromorphs the sting was present if the abdomen was like that of the worker, but absent when the abdomen was like that of the male. No definite relation was found between the superficial characters of the abdomen and its contained gonad. Testis and ovary might even be combined into one organ. Externally the male genital apparatus might be present and ovaries and oviducts exist inside.

Von Siebold attempted to account for the gynandromorphs by the assumption that an insufficient number of sperm entered the egg, so that part of it lacked sufficient quantities of the male element. It is true that recent discoveries (Nachtsheim) have shown that more than one spermatozoon does usually enter the egg, but we can not explain the results on this basis in the sense intended by von Siebold. Von Siebold gave no clear account of the varietal character of the male and female parts of the gynandromorph (see Boveri, p. 286), and in consequence it is not possible from his account to determine whether the male parts were like those of the Italian or German parent. It remained for Boveri, after 47 years, to attempt to make out, from the alcoholic remains of some of von Siebold's bees, the character of these parts.

In order to better present here the conditions in the gynandromorph, copies of Mehling's figures of the head of the normal bees are reproduced in text-figure 64, *a*, of the drone, *b* of the worker, and *c*



TEXT-FIGURE 64.

of one of the gynandromorphs of von Siebold's bees. The compound eyes of the drone are enormous in comparison with those of the worker, and meet above at the top of the head. The three simple eyes are, in the drone, forward near the middle of the "face", but on the top of the head in the worker. In the latter there is a tuft of long hair on the top of the head. In the gynandromorph copied in figure 64 the same approximate differences in the size of the compound eyes is seen on the male and on the female side. Two of the simple eyes on the drone side are low down, while the third, on the worker side, is at the top of the head, where a small tuft of hair is also present. The face of the worker is darker than that of the male, and the same difference in color is seen in the gynandromorph. The antennæ are larger in the drone, and this difference, too, is manifest in the gynandromorph, as is also the difference in size of the jaws. Many other differences

as striking as these are found in other parts of the body and come out equally well in the gynandromorph. Miss Mehling shows that the male and female parts may sometimes, however, be so intimately combined that a particular organ, such as a leg, may seem, on superficial examination, to be a blend of the two. A minute examination shows, however, as a rule, that such an organ is a piecework or mosaic of male and female characters.

It will be recalled that Boveri's hypothesis appealed to the phenomenon of partial fertilization. A belated sperm, sometimes failing to fuse with the egg nucleus before the latter divides, comes to combine with only one half of the latter. As a result, one of the first two segmentation nuclei contains only the maternal daughter nucleus, the other the combined maternal daughter nucleus and the entire sperm nucleus. The application of the results to the gynandromorph bees is obvious. If these are due to partial fertilization, then we should expect the male side to be like that of the mother's race—the Italian bee—because its haploid chromosome group came directly from the Italian mother's egg. *Vice versa*, the female side should show hybrid characters, or the Italian character if the Italian race dominates completely the German race. If the latter, both sides would then be alike and racially indistinguishable. Morgan's suggestion of polyspermy leads to the following explanation: If under unusual circumstances one (or more) of the spermatozoa should develop, the parts supplied by its nuclei would be haploid, hence male, while the other parts resulting from the combined nuclei would be female. The expected characters of the two parts of the gynandromorph would be the reverse of those called for by Boveri's hypothesis, for the male parts should be paternal on Morgan's view, maternal on Boveri's. The decision lies, therefore, in the character of the male parts of the gynandromorph. Boveri examined von Siebold's bees, some of which had been preserved in Munich, in order to get an answer to this problem, and reached the conclusion that the male parts are maternal. Hence the answer was in favor of his own hypothesis. We may now proceed to examine this evidence in detail and then see whether the hypothesis of chromosome elimination may not fit the facts as well as either of the alternative views.

After nearly 50 years in alcohol the Eugster gynandromorphs had lost so much of their color that a comparison with the racial pigmentation as seen in the living bees was impossible. Only after extracting the superficial pigment and dissolving away the external parts of fresh individuals of the two parental races was it possible for Boveri to make any reliable comparisons. Even then only a few individuals were available, because "an vielen Exemplaren das Abdomen nahezu farblos ist." Boveri confines his account to four specimens and in these takes only the head and abdomen into account.

The difference in the coloration of the heads of the males of *mellifica* and *ligustica* is in the prepared skeleton very slight, but Boveri thinks that the male parts of the gynandromorphs' heads are colored more like the same parts of the *ligustica*. Their abdomens show more striking differences, not only in the relative amount of deeper pigment, but in the pigment pattern as well. A comparison of the distribution of the pigment of the male side of the gynandromorph with the sides of the males of the two races seemed to him to show again that the closer match is with the *ligustica* type of pigmentation. The deep pigmentation of the ventral surfaces of the two races, especially in the males, offers more positive differences, especially as to color-pattern. The comparison shows here that when the abdomen of the gynandromorph is male its deeper color is more like the *ligustica* type, except when in places the male parts include or are replaced by female areas. Despite the fact that the comparisons that Boveri gives rest on rather a slender foundation, the evidence, so far as it goes, is clearly in favor of his interpretation of the nature of the male parts of the gynandromorphs. The well-known accuracy and carefulness of Boveri's work prejudices one strongly in favor of his opinion.

Boveri's evidence would seem, then, to settle the case in his favor were it not that another account appeared just before the publication of Boveri's paper (which he cites at length), based on observation of living[1] material—an account that leads to exactly the opposite conclusion from that reached by Boveri. Engelhardt described (1914) some gynandromorphs in which he stated that the male parts are dark brown (paternal) and the female reddish yellow (maternal). These gynandromorphs came from an Italian mother and a father belonging to a local (einheimischen) race. The case is parallel to the Eugster bees, and the only room left for doubt is the nature of the local race. The local race of the northern Caucasus, whence the evidence comes, is probably, according to Boveri, *Apis mellifica ramipes*. Here there is some more recent evidence that is important. Quinn has shown (1916) that when a yellow Italian queen is crossed to a gray drone of the Caucasus race the daughters (hybrids) and the drones are yellow like the Italian. This result indicates that the material used by von Engelhardt was suitable for giving differences in the gynandromorphs that could be used to distinguish the character of the male parts. It follows that von Engelhardt's results support Morgan's and not Boveri's hypothesis.

Since these views deal with paternal or maternal nuclei as wholes, it is immaterial whether the factor differences are carried by the sex chromosomes or by some other chromosomes, but when the third view comes up for consideration the question of which chromosome pair is involved is of vital moment. Let us see, then, how the hypothesis

[1] Or at least not alcoholic.

of chromosomal elimination applies to von Siebold's and von Engelhardt's gynandromorphs.

In the first place, it is important to understand that there is no conclusive evidence that the racial difference here involved has anything to do with one particular chromosome, or even with the sex chromosome. In bees the mother transmits her characters directly to her sons, as is the case in sex-linked inheritance of the *Drosophila* type, but in bees this form of inheritance is obviously due to the fact that the male develops directly from the unfertilized egg, hence must inherit *all* the maternal characters, whether in the sex chromosome or in the autosomes. The special sex differences are, of course, due to whatever it is that makes the egg a male or a female. In cases where the queen is heterozygous she may produce two kinds of sons, which is expected if the two races differ in one Mendelian gene,[1] but this would hold whether this pair of genes is autosomal or in a pair of sex chromosomes. If the two races differ in more than one pair of genes, more than two kinds of males are expected. The clearest evidence that we have in regard to what a hybrid queen produces is furnished by the recent work of Newell (1914) and Quinn (1916). Newell crossed a yellow Italian queen bee to a gray Carniolan drone. The daughters were yellow like the Italian, showing the dominance of that color. In the reciprocal cross, Carniolan female by Italian drone, the daughters were also yellow, but not as completely so as in the last. Whether this is due to modifying factors of some kind is not known. Quinn, as stated above, used Italian and Caucasus races, crossing both ways, and in both the daughters were the same, viz, yellow like the dominant Italian race. He also found that the F_1 daughters gave two kinds of drones and two only, which indicates that the factor difference is carried by a pair of chromosomes, but this evidence *alone* does not show that the pair is the pair of sex chromosomes, for any other pair would give in the bee the same result. However, when taken in connection with the gynandromorph results, the evidence becomes somewhat stronger that sex chromosomes are involved.

What, then, is the expectation on the elimination view? It is at once apparent that the elimination must involve a sex chromosome, for, otherwise, there is no reason to suppose that an autosomal difference would at the same time be associated with a difference in sex. In other words, the elimination hypothesis can apply here only if the chromosome that determines sex is the same chromosome that causes this racial difference.

Elimination of one of the sex chromosomes that carries the factor for *mellifica* would produce a cell containing only the *ligustica*-bearing chromosome, and all parts descending from that cell would be both

[1] It has been pointed out that the exceptions recorded by Cuénot may be due to drones coming from hybrid workers. (Morgan, 1909*a*, Am. Nat., XLIII.)

male and *ligustica*. This is the result which Boveri thinks is shown by the Eugster gynandromorphs. Conversely, if the *ligustica* chromosome were lost, all parts containing the descendants of this nucleus would be male and *mellifica*. This is the result that von Engelhardt claims to have found in his gynandromorphs. Thus both results are expected on the hypothesis of chromosomal elimination; each is equally possible. Boveri's hypothesis of partial fertilization explains only one case; Morgan's former view of sperm-nuclear development will explain only the other; the hypothesis of elimination will explain both, and for this reason is at present to be preferred. Moreover, since it is demonstrably the way in which gynandromorphs are produced in *Drosophila*, this hypothesis is more general than either of the earlier views.

There is a further implication in these cases of hybrid gynandromorphs in bees that can now be cleared up. The female parts of the gynandromorph are of hybrid origin. On any view, therefore, these parts are expected to be not necessarily like the mother (unless her character is the dominant one), but hybrid. If the *mellifica* color is dominant, then on Boveri's views the female side of the gynandromorph should be *mellifica*, but according to Newell the Italian yellow color is dominant, hence in half the Eugster gynandromorphs the male and female sides should have the same color. Perhaps this accounts for the astonishing failure on the part of von Siebold to mention the color differences in his gynandromorphs, since the superficial (the racial differences in color) color was often the same on the two sides. If this is the real situation, Boveri must have worked with a deeper color difference, one that is ordinarily not apparent. It is doubtful from his description whether he could determine if the female parts were *mellifica* or Italian or intermediate. He recognizes the difficulty, for he refrains from making any comparison between the female parts and those of the hybrid workers, but so far as he suggests any comparison it is with the pure *mellifica* type.

In von Engelhardt's case the male parts are described as darker, hence more like *mellifica*, while the female parts are described as lighter. Since Quinn shows that the yellow (lighter) color is dominant, the two sides should be different, hence the fact strongly supports von Engelhardt's interpretation. In fact, I do not see how we can avoid the conclusion that von Engelhardt's results are supported by much better evidence than are Boveri's own, if any such comparison must be made. Both are probably right, and the theory of chromosomal elimination not only accounts for both, but on that theory both kinds of results are expected.

If, as here suggested, both the Eugster and the von Engelhardt gynandromorphs are due to chromosomal elimination, it follows that there must have been also other gynandromorphs present that were not color-hybrids, but show the dominant color both in male and in female regions. In fact, these must have been as common as the

hybrid types. How can we account for this absence of all reference to such cases? It is to be recalled that Boveri actually studied only a few cases, stating that others were not sufficiently well preserved to show the hybrid differences between the parts. It should also not be overlooked that the more striking differences in color in the living hybrid bees would draw attention to these, while gynandromorphs colored alike on both sides would be overlooked. A census of all the gynandromorphs occurring under such conditions is necessary before it will be safe to conclude that these reciprocal cases did not occur. Boveri was, of course, only concerned with such cases as showed the maternal character of the male parts, and as such are expected in half of the cases, it would be natural to select these as illustrations of his theory. Until another survey of the entire output in such cases is recorded this test of the correctness of the elimination hypothesis can not be applied.

Wheeler (1910) has described a beautiful case of gynandromorphism in a mutillid wasp. The male half of the body is black and winged like the male, while the female half is rich red and wingless.

The ants are closely related to the bees, and sex determination appears to be in general the same, although there are some cases, apparently well authenticated, where unfertilized eggs have produced queens and workers as well as males. There were, prior to 1903, 17 cases of gynandromorphs known in ants which were brought together by Wheeler, to which he added, in 1914, 6 new cases. These show the same relations of parts seen in bees and call for no further comment. None were hybrids and furnish, therefore, no evidence for causal analysis.

GYNANDROMORPHS IN LEPIDOPTERA.

The group of Lepidoptera, including butterflies and moths, has furnished more gynandromorphs than any other group of animals, even more than the single species *Drosophila melanogaster*, if all butterflies and moths are taken together. It has been estimated that at least 1,000 cases of gynandromorphs have been recorded for this group.[1] Whether they are actually more frequent than in other insects

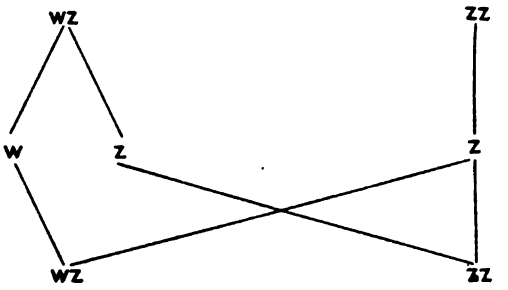
[1]Wenke (1906), summing up Schultz's reviews of 1898-1899, states that the 909 gynandromorphs (and hermaphrodites) brought together by the latter fall within the following species:

Species.	Individuals.	Species.	Individuals.
Smerinthus populi.....	67	Lycæna icarus.....	28
Saturnia pavonia.....	51	Bombyx quercus.....	24
Rhodocera rhamni.....	40	Ocneria dispar.....	23
Rhodocera cleopatra.....	34	Bupalus piniarius.....	16
Anthocharis cardamines...	33	Lasiocampa fasciatella...	15
Argynnis paphia.....	33	Limenitis populi.....	13
Lasiocampa pini.....	29		

or whether, owing to the striking character of their wings, they have more often attracted attention, is perhaps open to question. The differences between the coloration of the males and females in some species would at once arrest attention. On the other hand, in certain species and in certain hybrid combinations the number of gynandromorphs is so great that there can be little doubt that their occurrence here is directly related to the specific or to the hybrid nature of the insects.

Eleven more gynandromorphs of *Argynnis paphia* added by Wenke brings the total to nearly 1,000.

In regard to the chromosomal background, the situation is the converse of that in *Drosophila* and in nearly all other insects. The male has two sex chromosomes (text-fig. 65), which may we call ZZ, and the female one, Z, and another called W, corresponding to the Y of *Drosophila*. The genetic evidence in the case of *Abraxas* makes this view highly probable, and Seiler has shown in another moth that there is, in fact, such a chromosomal difference between the female and the male. As has been stated, in *Drosophila* the female combination XX is the basis for most of the gynandromorphs because the combination allows, through the elimination of one of the X's, the formation of parts with one X which is male. By analogy we should expect in Lepidoptera that the male combination ZZ would furnish the basis for the gynandromorphs of this group, since through elimination of one Z the female condition would arise.

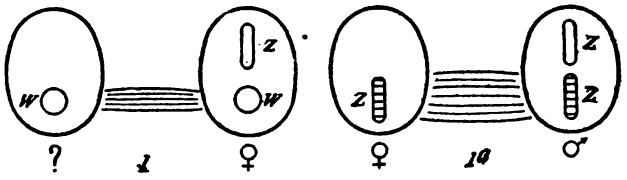


TEXT-FIGURE 65.

The most interesting case in the Lepidoptera is that of a hybrid gynandromorph in the silkworm moth, because here we know the genetic relation of the factors involved. Toyama obtained two bilateral gynandromorph caterpillars whose mother belonged to a race with a striped "zebra" pattern in the caterpillars and whose father belonged to a race with unicolorous white larvæ. Experiments show that in general zebra pattern is dominant to white. Neither is sex-linked. The left female side of the gynandromorph caterpillar was zebra, the right side white. If we attempt to analyze this case on the basis of Boveri's or of Morgan's earlier views—views based on the assumption that one or two nuclei determine male and female respectively—and assuming that, as in the bees, the male parts have one nucleus and the female parts the combined nuclei, then the result confirms Morgan's view and not Boveri's. But this interpretation does not

get to the bottom of the situation in the light of more recent work, for in moths it now seems probable that one Z sex chromosome (the equivalent in part of one nucleus) makes a female and two a male. There are, then, two kinds of ripe eggs, one with, the other without, a Z, and one kind of sperm, which is Z-bearing. There are six possibilities to be considered (see diagrams, text-figs. 66, 67, 68).

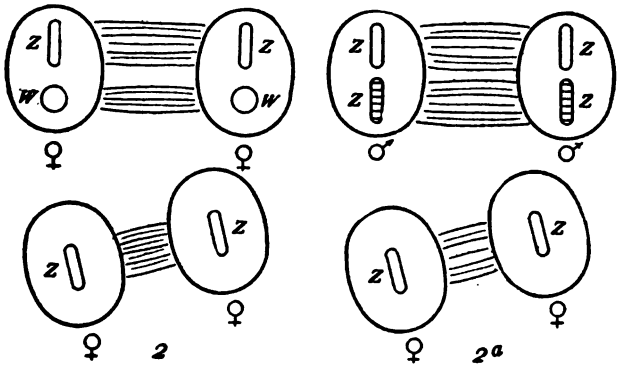
(1) On Boveri's view (text-fig. 66, *1*), if an egg *with* a W was the kind fertilized, then one half of the maternal segmentation nucleus should have no Z and would probably not develop, while the other



TEXT-FIGURE 66.

half of the egg nucleus, that united with the sperm nucleus, should have one Z and be both female and white. This explanation fails to account for the male sex of the side supposed to be without a Z and for the presence of zebra on that side.

(1*a*) On Boveri's view (text-fig. 66, *1a*), if an egg *with* a Z were fertilized by a sperm (bearing Z), then both the male and female sides should be zebra, which is contrary to evidence.



TEXT-FIGURE 67.

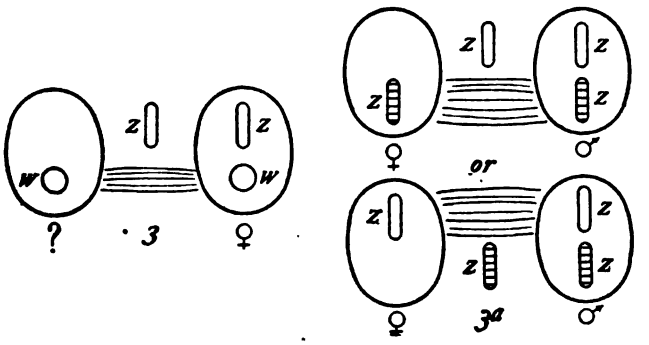
(2) On Morgan's earlier view (text-fig. 67, *2*), an egg *with* a W fertilized by a sperm (bearing Z) should give female parts from the combined nuclei which would be white. The sperm nucleus alone would also give female parts which would be plain. The result is a mosaic, but not a gynandromorph.

(2*a*) On Morgan's view (text-fig. 67, *2a*), if an egg *with* a Z had been fertilized by a Z sperm, all male parts (ZZ) should be zebra. The female parts would be plain, which is again contrary to fact.

(3) On the elimination hypothesis (text-fig. 68, *3*), an egg *with* a W fertilized by a sperm (Z-bearing) should produce a female (ZW). This gives no chance to produce a male side (ZZ) by ordinary elimination. If by somatic non-disjunction (ZZ or no Z) it is not evident that the no-Z part would develop, and if it did, why it should be plain.

(*3a*) If an egg *with* a Z had been fertilized by a Z sperm (text-fig. 68, *3a*), a male (ZZ) would result from which, by elimination of a Z chromosome bearing the white gene, would produce female parts that are zebra and male parts that are also zebra, which is contrary to the actual conditions in the gynandromorph. If the other chromosome should be eliminated, viz, the one bearing the zebra gene, then the male part would be zebra and the female part would be plain.

It is evident that this case can not be explained in any of these ways, even though it be assumed that the color-factors are carried by the sex chromosomes. And if we do treat the color-factors as sex-



TEXT-FIGURE 68.

linked, then they can not be the same zebra-white pair of factors described by Toyama in other crosses which are clearly not sex-linked. To apply the above view tactily takes for granted that the zebra-white pair is not the same pair referred to in other crosses. If it is not, then we are not obliged to assume that zebra is dominant to plain. If plain is assumed to be dominant over zebra, the gynandromorph can be accounted for by Boveri's hypothesis or by elimination. Possibly one might try to find an excuse for such an evasion by pointing out that Toyama states that the two gynandromorphs appeared in a cross between a striped French race with yellow cocoons and a common Japanese race with white cocoons, and that this is not the same cross as that which he described in the body of his paper, where he states that the striped race had *white* cocoons. On the other hand, both Coutagne and Kellogg, according to Tanaka, have found that striped is dominant to plain, and although I can not find that they have made exactly the same cross as that which yielded the gynandromorph, nevertheless the cumulative evidence is strongly in favor of the view that zebra is both dominant and not sex-linked. It is clear, then, that

we must search for some other kind of explanation for Toyama's gynandromorphs. Fortunately, Doncaster's observation on the eggs of a race of *Abraxas* gives us a clue to an explanation. Doncaster, as stated on page 20, found occasionally an egg containing two nuclei, each nucleus being about to be fertilized by a separate spermatozoon. Now, if in Toyama's case the zebra mother was heterozygous, one of the two nuclei in question might contain a Z chromosome and an autosome with a gene for plain color (Z and white), while the other nucleus might contain a W chromosome and an autosomal gene for zebra (W and zebra). Two sperms of the father, each with a white-bearing autosome, each fertilizing one egg nucleus, would give a white male side (Z, white; Z, white) and a female zebra side (W, white; Z, zebra). This seems the most probable interpretation.

There is still another possible explanation of Toyama's gynandromorphs, viz, that the male parts have come from the fusion of nuclei derived from two (or more) spermatozoa. Pairs of such nuclei would give ZZ cells that would be male and paternal. It is true that Herlandt and Brachet find in the frog that sperm nuclei do not fuse in the egg, but they attribute this to the cytasters that keep them apart. If in the moth (and bee?) the cystasters are less well developed, contiguous nuclei might sometimes fuse.

Another moth, *Abraxas*, has been extensively used by Raynor and Doncaster in genetic experiments. The characters in question (*grossulariata* versus *lacticolor*) show sex-linked inheritance and should furnish interesting evidence as to the nature of gynandromorphs in moths.

Quite recently Doncaster has reported two gynandromorphs of *Abraxas* that arose in a cross between these two types. The first case arose in a cross between *grossulariata* female by *lacticolor* male. The normal expectation for this cross is: *grossulariata* males and *lacticolor* females. There were produced 24 *lacticolor* females, no *grossulariata* males, and one gynandromorph that was *lacticolor* but mixed in certain parts. The absence of males is apparently connected with an exceptional chromosomal condition in this family (viz, 55 chromosomal line) of such a sort that all the fertilized eggs lacked a chromosome, the single Z passing out into the polar bodies in all or nearly all cases. The main characters of this gynandromorph are "the right antenna is male, the left female, and the frenulum of the left wing is of the male type and well developed, that of the right male but imperfect. In the external genitalia the chief points are that the uncus, anus, and ovipositor are each divided; the right vulva is not unlike that of a normal male, the left side is abnormal and has attached to it a second anus and half of the ovipositor," etc. Doncaster sums up the chief peculiarities of this moth as follows:

"(1) That though predominantly male, it has the *lacticolor* character which, from its parentage, should be confined to females; (2) throughout the body

the right side is male, the left imperfectly developed, a tendency towards the female type. . . . . The internal genital organs were, as far as is known, imperfectly developed male organs."

A theoretical explanation of the case, based on the chromosomal peculiarities of the line, is as follows: Since practically all eggs had but one Z chromosome before polar-body extrusion and lost it at their formation, few males arise as Doncaster has shown, and even if Z should exceptionally remain in a ripe egg it would carry the gene for *grossulariata*; hence any male coming from it would be *grossulariata*. Only then by the sperm bringing two Z's into a Z-less egg could a *lacticolor* male arise. Such an abnormal sperm could arise in any male by primary non-disjunction, or by secondary non-disjunction from a ZZW male, *i. e.*, by the two Z's of the spermatogonia both passing to the same pole at one of the maturation divisions. If this happens, a *lacticolor* male is expected. The appearance of femaleness in certain parts of the left side must, then, be referred to an elimination of one of the Z's at some early division.

Doncaster's second case can be explained as a simple case of chromosomal elimination. A *grossulariata* female, by *lacticolor* male, gave 11 *grossulariata* males + 11 *lacticolor* females + 1 gynandromorph whose anterior parts are male (including the wings to some extent), and whose posterior parts are female. Here the normal proportion of males to females, and the expected distribution of color to them, shows that the female was normal as to her chromosomes. If we assume, then, that a Z-bearing egg was fertilized by a normal Z-bearing sperm, the result should be a normal *grossulariata* female heterozygous for *lacticolor*. Elimination of one of the paternal Z's would give a *grossulariata* male in the anterior region coming from the ZZ nuclei and a *grossulariata* female posterior part coming from the single Z nucleus. The second case is comparable in every way with the cases of *Drosophila* and allows an extension of the theory of chromosomal elimination to the group of moths, in line with the other critical cases described above. Doncaster's first case must also appeal in part to the same hypothesis, but it is more complicated, since another exceptional phenomenon must have first occurred. This first process gives a *lacticolor* male when a *grossulariata* male or no males at all are expected. It is only that elimination later happened to take place in this individual that it comes to be considered in this connection. In other words, there is no necessary connection between the two events, so that the non-disjunction phenomenon does not in reality complicate the elimination explanation. The two are quite independent. It should be pointed out that such exceptional males due to non-disjunction are known to occur in *Abraxas*.

Another gynandromorph in *Abraxas* (Tutt, 1897) involves varieties *A. ab. suffusa* and *A. ab. obscura*. Since the genetic relation of these

characters to the type *grossulariata* are not known, nor the parentage of the individual, no analysis of the case is possible. A third aberrant type, *nigra*, has given a striking bilateral gynandromorph with *grossulariata* (figured by Cockayne). The genetic evidence in regard to this type obtained by Punnett fails to show that the character is a simple Mendelian one, so that this evidence is not available for analysis.

The most remarkable mosaics of male and female characters are shown by hybrids of the gipsy moth, *Porthetria dispar* and *japonica*. These mosaics have been described by several observers (Wiskott. 1897, Brake, 1907–1910; Brake and C. Frings, 1911; Goldschmidt, 1912–1917; Poppelbaum, 1914). We owe to Goldschmidt not only a most complete account of the hybrids between these two varieties, but of hybrids involving several Japanese local varieties of this moth. In the latter crosses a most astonishing series of mosaics come to light, not as sporadic occurrences, but as regular phenomena of the cross. In his earlier work Goldschmidt called these mosaic forms gynandromorphs, but his later work shows, he thinks, that they are different from gynandromorphs; he now calls them intersex forms.

The normal males and females of the gipsy moth differ not only in the characteristic sex differences of this group, but in other secondary sexual differences also. The Japanese varieties show these same sexual differences, though both sexes differ in color and in a few minor points from the European species. *Japonica* female by *dispar* male gives equal numbers of daughters and sons that are normal as to sex, but the reciprocal cross, *dispar* female by *japonica*, gives normal males and intersex females in equal numbers.

These intersexual females from different crosses show a wide range in structure, in color, and in behavior, from almost normal females at one end of the series to forms that externally are about like the normal male. Not only are the wings colored like those of the normal male (with occasional flecks of white like the female), but the antennæ, the hair, the size, the genitalia, and the gonads themselves are mosaics of male and female and intermediate conditions also. These relations are more interesting where crosses involving different Japanese races are compared. When a race, Jap. G male is crossed to Jap. K female, all F_1 daughters are *slightly* intersexual. When a race, Jap. H female is crossed to Jap G male, the daughters are somewhat more like the males, but the instincts are still female and they attract males. The copulatory organs are so changed in the direction of the male that mating is unsuccessful, and eggs can not be laid, although the characteristic hairy sponges are made. When a race, Eur. F female is mated to Jap. G male, the daughters are "more than half-way between males and females." The secondary sexual characters are almost male. The instincts and behavior are about intermediate between those of the two normal races. Males are scarcely attracted

or not at all, and no mating occurs. The copulatory organs show the strangest combinations of the male and female type, but there are still typical but rudimentary ovaries left. When the race, Jap. X female is crossed to Eur. F male, a still *higher* degree of intersexuality appears. Externally the daughters are "almost indistinguishable from true males." The instincts are entirely male and the moths try unsuccessfully to mate with females. The gonads look like testes, but in sections show a mixture of ovarian and testicular tissue. A step further and the daughters would be transferred into males.

The next cross gives this final stage. When Jap. O male is crossed to any race of European female, only males are produced, *i. e.*, all the daughters become sons.

The reverse picture is given by those combinations in which the intersexes are sons partly changed over into daughters, a condition that Goldschmidt terms male intersexuality. The wings are generally streaked and in the extremest type only a few brown spots appear on the wing-veins. The testis may contain some ovarial tissue, but the changes in the gonads do not appear to run parallel to those seen on the surface.

The explanation that Goldschmidt offers for these intersexes is entirely different from the explanation that is demonstrated for the gynandromorphs of *Drosophila*. He accepts in part the chromosome theory of sex determination and applies it to the present case on the basis that the female is heterozygous for the sex chromosome Mm, and the male homozygous MM. In addition, however, Goldschmidt adds another set of sex-determining factors that he calls FF (inclosing them in brackets), which he locates in the cytoplasm, that is, outside the chromosomal mechanism. These factors do not segregate (the desirability of two F's is therefore not apparent) and are transmitted from the female to her sons and daughters alike. The FF factors stand for femaleness, which apparently includes the eggs, ovaries, secondary sexual characters, and genitalia, in fact, all parts associated with the female. The sex of a given individual is dependent on the balance struck by the activity of the factors Mm and FF, one in the chromosomes and the other in the cytoplasm.

The FF factors are supposed to be located in the cytoplasm because if a certain numerical value is assigned to the egg, this value adheres to the maternal line, no matter which sex chromosomes are introduced from the male side in successive generations. If the factors for femaleness were carried by the male and like other paternal characters influence the cytoplasm, their value would be affected by the kind of males that were employed; but Goldschmidt has shown that his results work out on the assumption that no such effects need be postulated.

There is, however, another way in which the inheritance of certain factors along the maternal line may be treated. Goldschmidt has himself admitted this as a possible interpretation, although he has

adopted the cytoplasmic agency. In moths there is present, in certain species, a W sex-chromosome analogue of the Y of *Drosophila* that is always carried along the female line. If this chromosome carries factors it becomes one of the conditions of the result and the eggs will always be under its influence, and hence differ from the spermatozoa by a constant difference. This assumed difference might account for the fact that in reciprocal crosses the results differ and certain phases of the inheritance consistently follow the egg. There would be no theoretical objection to calling this difference "factors for femaleness." If crossing-over took place between the W and the Z chromosomes, however, this constancy would disappear. Until critical evidence can be obtained, such as the loss of the W chromosome from a line, there is no way of proving or of disproving the cytoplasmic *versus* W-inheritance hypothesis.

In regard to the numerical values that Goldschmidt assigns to M and F, it is obvious that these are from the nature of the case arbitrary, such values being assumed as will give a consistent interpretation. Whether this mode of treatment has the advantage of a *quantitative* procedure, as claimed, is not so obvious, for the values are simply assigned to the data and are not given by any outside common measure, such as the chemist or physicist uses in quantitative work. If, then, the values are only numerical assumptions, the treatment is not, as Goldschmidt thinks, lifted above the symbolic handling of the problem of heredity, but stands on the same footing as all Mendelian procedure. If the numerical values assumed give consistent results when tested in other crosses where other numerical values have been assigned, there is an undoubted value in handling the problem in this way quite irrespective of the question as to what a quantitative treatment may mean.

As stated, Goldschmidt interprets his results as depending on a quantitative relation of the opposing factors for femaleness and for maleness. If the quantitative difference between the factors is sufficiently great in one direction the individual is a male; if in the opposite direction it is a female. If the difference is not sufficiently great either way an intersex develops. If the quantity of the female factors were greater at the beginning a female intersex results; if the quantity of the male factor were greater at the beginning a male intersex develops. Both kinds of intersexes grade in different crosses all the way from nearly normal females to nearly normal males or from nearly normal males to nearly normal females. In each series the sequence in which the characters change towards those of the opposite sex is the reverse of the order in which they develop in the individual. "The last organs to differentiate in the pupa and the first to be intersexual are the branching of the antennæ and the coloration of the wings. The first imaginal organ differentiated in the caterpillar and the last in the series to be changed toward the other sex is the sex-gland. And if we apply

this law even to the minute parts of a single organ, like the copulatory organ, we find it also to apply, as will be demonstrated later. Now, this is the fact which, in connection with the others, enables us to formulate a definite physiological theory of sex-determination."[1] Goldschmidt sums up the situation in the following statement:

"First, we recognized that the different effects of the same sex-factors in different combinations can be understood only by assuming a quantitatively different action; or, expressed in concrete terms, that the active substances, which we represent as factors, are present in different but typical quantities. Second, we were obliged to assume that these substances are distinct for each sex. Third, we realized that in the action of these substances a time factor is involved, which is definitely proportional to the quantities of the factorial substances. From these facts only one conclusion can at present be drawn: that the sex-factors are enzymes (or bodies with the properties of enzymes) which accelerate a reaction according to their concentration. . . . ."[2]

If the nature of the character is dependent on the relative quantities of the male-producing enzyme called andrase and of the female-producing enzyme called gynase, the question arises how intersexes that are mosaics would ever arise, for there is no obvious reason why the relative concentration should ever change in the course of development as Goldschmidt must assume that it does change. Still less is it clear, when the difference in the concentration is less than a given critical difference (Goldschmidt's definite minimum value *e*), why the enzyme that starts with a lesser concentration should always overtake the other quantity, no matter which one starts below. Until this critical point is explained all the speculation that Goldschmidt brings to bear on the question only seems to cover up the difficulty rather than to clear it up. Goldschmidt appears to have overlooked this difficulty and sets up the opposite one, viz, that it is difficult to see why every gipsy moth is not an intersex. He meets this supposed difficulty by the consideration of the rate of development of the insect. Whether his answer to this difficulty is valid or not, it does not seem to meet the difficulty which to us seems the real one.

Even were it established that many of the changes in embryonic and larval development are due to enzymes—a point that we are far from wishing to dispute—it need not follow that the segregating genes that give rise to them are also these same enzymes. To treat these half-way stages as the genes themselves is at present not without danger, because even if the genes are enzymes it by no means follows that the quantity of the gene is to be measured by the product of the enzyme arising from it.

In his latest communication Goldschmidt states his belief that the sex-factors in the different races of gipsy moths are multiple allelomorphs and compares them to the series of factors that Castle has

[1] Goldschmidt. A Further Contribution to the Theory of Sex. (*Journ. Exp. Zool.*, vol. 22, No. 3, April 1917, p. 597).
[2] *Ibid*, p. 598.

found in his series of hooded rats. So far as we know, the conclusion that Castle's series of characters are mainly due to multiple allelomorphs is far from being established; on the contrary, we are inclined to think that his evidence indicates that he is dealing mainly with a case of multiple factors. Some of the evidence that Goldschmidt himself furnishes for the gipsy moths is perhaps also capable of interpretation in the same way.

Goldschmidt has shown in some detail that the characters or organs of the intersexes, such as the wings or external genitalia, are mosaics—*i. e.*, relatively large segments or pieces are entirely male or female. In the case of the wings there is no obvious regularity in the mosaic pattern, for the right hind wing may be entirely different from the left hind wing, and the male parts of the right wing do not by any means correspond to the male parts of the left wing, nor does either conform strictly to any underlying structure, such as the veins. In so far, then, as each part is strictly male or female and not a blend of both, the gipsy-moth intersex is like the *Drosophila* gynandromorph. The results are, however, unlike the *Drosophila* gynandromorphs in that in the gipsy-moth hybrids the phenomenon must occur very frequently. Baltzer has shown for certain sea-urchin hybrids that when the cross is made one way there is always an irregular (?) elimination of chromosomes, and this result invites at least a comparison with the gipsy hybrids. A solution of the case of intersexes in the gipsy moth could probably be reached by the discovery and study of sex-linked characters.

Several gynandromorphs of *Colias* have been described (see Cockayne), but of unknown parentage. In the moth *Algia tau* also several gynandromorphs have been recorded, but the published evidence known to us does not give any clue as to their origin.

As has been stated, the great majority of gynandromorph Lepidoptera are not hybrids, but show the secondary sexual characters of the male on one side and the secondary sexual characters of the female on the other. There are, however, a few gynandromorphs in this group that show racial or specific differences along with the male and female characters. Amongst these only a few have a known ancestry, and amongst these again it is seldom known whether the characters exhibited are sex-linked or not. Even if they are sex-linked the evidence fails to discriminate between a result that depends only on sex-chromosomal differences and a result that depends on a full chromosome group. A search through most of the available literature has brought to light only a few cases that bear on the theories that have been already discussed. Nevertheless, it is probable that a more thorough search through the voluminous literature might furnish more of the critical evidence desired. It is not improbable that entomologists who have made varietal crosses may be able to supply some of the needed data.

From the elaborate list of gynandromorphs published in 1896 and 1897 by Schultz, and from the admirable résumé by Cockayne in 1915, the following cases have been chosen as the most instructive ones on record.

Wheeler (1915) describes a gynandromorph from a cross of *Smerinthus ocellatus* by *Amorpha populi* (*hybridus*). The right side is female, the left side male.

"The left wings are pinkish, as in *ocellatus*, while the right wings are entirely gray. The eye-spots of *ocellatus* are well developed on both wings, as is also the red basal patch of *populi*. Right antennæ like female *populi*, left like male *ocellatus*. Right half of body light gray, left half brownish gray."

Since both sides of the body show some characters that belong to both parents, it is highly probable that parts of both parental nuclei are present on both sides of the gynandromorph.

Briggs (1881) has also described a hybrid gynandromorph showing the characters of *Smerinthus ocellatus* and *populi*—right side *ocellatus*, left side *populi*. A figure is given, but no description. Whether from the figure it would be possible to determine whether some characters of both parents are present on both sides might no doubt be determined by an expert, but the all too brief text gives no information.

Harrison crossed *Ennomos subreguaria* male by *E. quercinaria* female, and obtained many hybrids that were "practically the mean of the parents, except that they leaned in the color, both of the head and body and possibly in the general structure of the warts and tubercles, to the male parent." In describing one of these, Harrison says:

"At first sight it is merely a male specimen with the left anterior female. Dissection and close examination betray much more interesting characters than that. The genitalia (fig. 4), although nearly so, are not quite purely male; the right lobe of the uncus is replaced by a fully developed right ovipositor or lobe, while the gathous on the same side is greatly disturbed, and acts as if it were homologous to the female directing rods. In addition, whilst the coloration of both sides of the body is male, the shape of the right wing is female."

Harrison points out that "whilst the majority of the characters of the right side were female the color was wholly male." It appears from this description that hybrid characteristics appeared throughout, which indicates that other chromosomes than the sex chromosome were involved on both sides; but since so small a part was distinctly female, it is not entirely clear that the hybrid coloration affected this part too. He states (as above) that "while the majority of characters of the right side were female the color was wholly male." Apparently by male he means hybrid male coloration, and if so the case is instructive.

Harrison obtained another aberrant individual from this cross. He states that "the right being exactly that of a normal hybrid, whilst

the left side is pure *subregnaria*." The text leaves it uncertain whether the individual is a gynandromorph, although this appears not to be the case, for while the former specimen is classified as a gynandromorph, this one is put into a separate paragraph entitled "Asymmetrical specimen." Its genitalia are said to "present the same division of characters as those exhibited externally, as may be seen from figure 8, which shows the furca and the penis, the left side being that of the hybrid, whilst the right is evidently *subregnaria*, conforming itself to the structure of the left."

Harrison explains the result as due to two spermatozoa entering the egg, the nucleus of one of which conjugated as usual with the egg-nucleus, but the nucleus of the other, instead of degenerating, gave rise to the nuclei determining the right side of the body, which would then be pure *subregnaria* and differs from the hybrid left side, which resulted from the conjugation of nuclei derived from two different species. Insofar as one side is purely paternal, this case is in line with Morgan's hypothesis of multiple fertilization and does not conform to Boveri's view. On the other hand, there is the same cytological difficulty here as encountered in Toyama's case, namely, that in Lepidoptera the male is the homozygous individual. A single nucleus should give rise, therefore, to a female, but here probably both sides, and certainly the pure *subregnaria*, side is male.

The hypothesis of elimination will not help out here, for even if a *quercinaria* daughter chromosome was the one lost, the single sex chromosome should give rise to female parts. On the other hand, one of the alternative views suggested above for *Abraxas* covers this case, viz, the view that an egg had two nuclei or that several spermatozoa entering and fusing in pairs gave rise to the male parts.

Cockayne (1916) described a hybrid gynandromorph that came from a cross of *Amorpha ocellatus* male by *A. populi* female. It was male on the right side and female on the left. Although the wings did not expand, it was evident that on both sides the specific characters were intermediate between the two parents. The insect had neither ovary nor testis, but the external genitalia were male on one side and female on the other.

Vasseler described a bilateral gynandromorph of *Argynnis paphia* in which the left side was male and *paphia*, and the right side was female and *valesina*. The latter is a characteristic varietal form. The result can be explained by dislocation of the sex chromosome on the basis that the factor of *valesina* is sex-linked and that it is recessive.

According to Rudolphi,[1] a gynandromorph was sent to McLeay from Rio de Janeiro, var. *Papilio laodicus* on the female side and *P. polycaon* on the male side. Dr. F. E. Lutz has been good enough to look up

[1] Rudolphi, D. K. A., Abh. phys. klass. Konig. Akad. wiss., Berlin, 1825. See Trans. Linn. Soc., XIV, p. 584.

for me the history of the "species" question. The following note I owe to him:

"*Papilio androgeus* is quite variable and, furthermore, shows sexual dichromatism. Three varieties are accepted: typical *androgeus* (Colombia to Trinidad, Guianas, Amazon, southward to Bolivia and western Matto Grosso), *epidaruus* (Mexico to Panama, Cuba, Haiti, and Saint Lucia), and *laodocus* (Brazil and Paraguay). The name *polycaon* has been used by authors for each of these forms and has been applied to both males and females. The name *laodicus* has usually (always?) been applied to the female. It seems probable that the specimen in question was an ordinary gynandromorph of *Papilio androgeus laodicus*."

Cockayne has discussed at length the evidence showing that gynandromorphism is commoner in certain species than in others, and reached the conclusion that this is not due, in several cases at least, to the more striking characters involved, but rather to some peculiar defect in the sex-determining machinery of these species. Moreover, there appears to be good evidence favoring the view that in certain families the number of gynandromorphs is greater than in the race as a whole. The cause of this "inheritance" is obscure. Possibly these are cases of intersexuality rather than of true gynandromorphism.

The evidence is more certain that gynandromorphs are more common in certain hybrid combinations than in the pure parent species involved in the cross. Whether such combinations are generally due to the greater liklihood of chromosomal elimination—a view that would seem *a priori* possible—or to "partial fertilization" or to polyspermy can only be determined when more definite material is obtained that furnishes opportunity for genetic evidence.

GYNANDROMORPHS IN OTHER INSECTS.

The scarcity of gynandromorphs in other groups of insects is probably due in part to the absence of conspicuous differences between the male and female in such groups as beetles or to certain groups being less collected or observed than others. We have made no attempt to search out in the literature all references to gynandromorphs. Occasional references to gynandromorphs in earwigs, Orthoptera, beetles, and bugs are to be found in the International Catalogue.

GYNANDROMORPHS IN SPIDERS.

In a recent paper J. E. Hull has brought together the few cases of gynandromorphs in spiders that are known. The best example is that described by Kulezynski that is male on one side and female on the other. Another described by Falconer was also male on one side, female on the other. Another gynandromorph described by the author (Hull) is male and female anteriorly and female and male posteriorly (quadripartite). Three other cases of bilateral gynandromorphs have

been reported, according to Hull, and one or two other gynandromorphs incompletely described.

Most of the gynandromorphs in spiders belong to one family. Thus amongst the 232 species of British Linyphiidæ there are seven gynandromorphs known, while amongst the 377 other species only one. Hull estimates that gynandromorphs are nine times as frequent in the Linyphiidæ as in all the rest taken together.

Since the male is heterozygous for the X chromosome in spiders the results may have the same explanation as in insects, but since no hybrid-gynandromorphs have been found it is impossible to do more than point out a possible solution.

GYNANDROMORPHS IN CRUSTACEA.

The frequency of bilateral gynandromorphs in insects is in marked contrast to the almost total absence of such types in the large group of Crustacea. It is true that in the latter there are examples of intersexual individuals, but it is not clear whether these come under the same category as the gynandromorph insects or are special cases more like hermaphrodites.

It may be of interest to observe in this connection that in the Crustacea no sex chromosomes have as yet been discovered, but it may be replied that this may be due to the well-known difficulties of technique rather than to a real difference. However this may be, there are certain well-ascertained facts about some of the Crustacea suggesting that the condition of hermaphroditism is, so to speak, nearer the surface in the sense that the swing towards one sex or the other in a given individual is brought about more readily by age or environmental conditions than in other groups where a change is more difficult because the internal hereditary factor differences prevail over ordinary external or age differences. For example, in the group of cirripeds hermaphroditic species and species with separate sexes exist, as well as species related to hermaphroditic species in which the females have complemental males. It has been suggested that these males are themselves only those arrested females or hermaphrodites that settle down and become parasitic on the larger sessile females; in other words, that these males had the potentiality of becoming females if they had chanced to lead a different existence. There are families amongst the isopods that are hermaphroditic. Certain species of amphipods are said to be males when young, females when older. Eggs have been found at certain stages intermediate in size between the small male-producing eggs and larger female-producing eggs.

The transformation of some of the secondary sexual characters of the male into those of the female in certain parasitized crabs has a bearing both on the relation of these characters to the sex-glands and possibly also on the causes that determine sex in the Crustacea.

Giard, and later Geoffrey Smith, have described the changes that take place when crabs are parasitized by *Sacculina* and other parasitic crustacea. When the male spider-crab *Inachus dorsettensis* is parasitized by *Sacculina* the abdomen becomes wide like that of the female, and its posterior appendages, that are absent in the male, develop and become somewhat like those of the female. The chelæ likewise come to resemble those of the female. The testis, which may not be affected at first, may later degenerate to some extent, and in one case after the parasite had fallen off the regenerating testis produced eggs. It was formerly supposed that the degeneration of the testis might be the cause of the change in the secondary sexual organs, although no such relation between gonad and soma is known to exist in this group; but the work of Geoffrey Smith seemed to him to suggest that the results are directly caused by the parasite itself by stimulating the formation of fatty substance whose presence in the blood may cause eggs to develop and the secondary sexual organs of the female to appear. In other words, "the crab comes to resemble a female because the physiology of its body-tissues has been changed from the male to the female type" (Doncaster). Whatever the explanation may ultimately be found to be, the fact of the change is important. The result falls into line with the other evidence concerning sex determination in the Crustacea, viz, that maleness and femaleness are not so fixed by internal genetic factors if such exist, but that the balance may be shifted by other agents as well. A parallel case is known in the Andrenine bees, parasitized by another insect, *Stylops*. According to Perez, the stylopized males come to resemble in certain respects the females, and inversely the stylopized females the males. The sex-glands are not always affected. If in bees as in moths the secondary sexual characters are independent of the gonads, the effect of *Stylops* must be either directly on the host or through a change in its metabolism. W. M. Wheeler has described stylopized American wasps of the genus *Polistes*. No change in the secondary characters takes place, at least not to any marked extent.

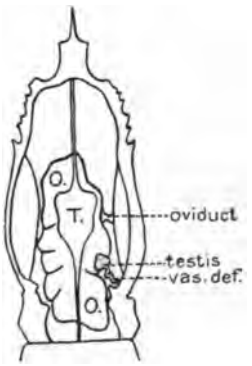
The decapods have, as a rule, males and females sharply distinguished, although the females of *Gebia major* have only ovaries, the males have, behind the testes, ovaries more or less developed. A crab, *Lysmata seticaudata*, has as a rule both ovaries and testes, with their ducts. Several hermaphroditic crayfish have been described, especially by Faxon (1898) and Hay (1907). One (text-figure 69) had ovaries on both sides, also on the right side a testis (without sperm) and a vas deferens. It had the external characters of a "first form" male except for the openings of the oviducts on the third pair of legs. It appears, at least in the genus *Cambarus*, that hermaphroditic individuals are females which, "owing to some ambiguity of the formative cells in the embryo, have developed to a greater or less degree the

characters of the opposite sex. The condition is a very rare one and is usually shown in the external organs only."

In the Philosophical Transactions for 1734 is a full account of a bilateral gynandromorph lobster by Dr. F. Nichols. The drawings of the external parts show that the animal is female on the right side and male on the left. Dissection showed an ovary with eggs on the right side, and a testis with vas deferens on the left. This case is exactly like the bilateral gynandromorphs of *Drosophila*, and is the only case known to us of a strictly bilateral type of gynandromorph in the group Crustacea.

Olga Kuttner (1909) found a wild individual of *Daphnia pulex* that had some male characters on one side but had two ovaries. Twelve broods were produced and in nearly every brood some individuals were mixed gynandromorphs, but nearly all were predominantly female.

A similar case has been recorded by Banta for *Simocephalus vetulus*, (1916). In a pedigreed strain there "suddenly appeared" a large number of "sex intergrades—males with one or more female secondary sex characters, females with one to several male characters, and some hermaphrodites with various combinations of male and female secondary sex characters." The more extreme intersex individuals fail to propagate; others, less modified, reproduce. By propagating from female intergrades mixed broods of males, females, and intergrades are obtained. The noteworthy point here is that the intergrades are mosaics rather than blended forms of the two sexes.



TEXT-FIGURE 69.

GYNANDROMORPHS IN MOLLUSCS.

The molluscs, like the Crustacea, contain a number of hermaphroditic species, but there are also species with separate sexes. Here, too, cytological study has failed as yet to demonstrate sex chromosomes. One species of *Crepidula* is male in the juvenile state and female in older individuals, at least when certain external conditions are fulfilled. Gould has recently shown that when young males are placed in the vicinity of large females the males absorb their testes and genitalia (ducts and penis) and develop ovaries and oviducts. This case recalls in many ways the conditions in *Bonellia* as described by Baltzer. If the embryos of *Bonellia* are isolated they become sexual females without showing the male stage. If, however, the embryo, when ready to settle down, comes to rest on the proboscis of a female it develops into a rudimentary male. A few embryos in cultures may show intermediate or rather hermaphroditic conditions. The cirripeds referred to above appear, according to one interpretation, suscep-

tible of similar modifications, according to whether they remain free or become parasitic on a female.

The conditions in Crustacea and molluscs seem to show that, in some cases at least, the animals are essentially hermaphrodites and that external conditions and age are important factors in determining the sex of the individual. These cases recall the phenomena shown by many flowering plants where at one stage or under certain conditions the male organs develop, under other conditions the female organs. If in such cases sex-determining genes are present, their influence may be readily overcome by external agencies or by age itself, which is in a sense a condition in which some part of the body (through its output) acts as an external agent to other parts.

GYNANDROMORPHS IN ECHINODERMS.

Cuénot (1898) and Delage (1902) described each a mature starfish (*Asterias glacialis*) that had small patches of testis (with sperm) in the ovary, and Buchner (1911) has recorded a similar case. Herlandt has recently described a sea-urchin (*Paracentrotus*) that had three normal and one "dark" testes and a large ovotestis with functional ova and sperm. Artificial fertilization with the products of this ovotestis was successful, and the larvæ were normal. Since Tennant has shown for the sea-urchin that the female is homozygous and the male heterozygous for the X chromosome, these cases can be easily explained on the hypothesis of elimination of an X chromosome—the resulting parts being male.

GYNANDROMORPHS IN VERTEBRATES.

The group of vertebrates shows as a rule sharp separation into two sexes, but the evidence relating to the factors involved is often so little known that the group as a whole is difficult to handle. In one subdivision, the birds, the female is the heterogametic sex with regard to sex chromosomes, while in mammals, certainly in man, it is the male that is heterogametic. The contrast here is the same as that in insects, where the moths resemble the birds and the flies man. In some of the lower groups there are evidences of hermaphroditism or of transitory sex conditions. It becomes necessary, therefore, to take up the different groups independently.

GYNANDROMORPHS IN FISHES.

Myxine, according to Cunningham and Nansen, is male when young and later becomes female. In the young the anterior portion of the testis is male, the posterior female; the testicular part atrophies after it has functioned as a testis. But the later results of the Schreiners indicate that while young *Myxine* is a true hermaphrodite as far as the histological structure of the glands is concerned, it is not so func-

tionally. They believe that any one individual after passing through this stage becomes definitely either male or female, although certain individuals remain sterile, neither alternative being realized (quoted from Caullery, Les Problèmes de la Sexualité, 1913, p. 53.)

A gynandromorph was described in 1914 by Vayssiere and Quintaret in one of the sharks, *Scyllium stellare*. The left pelvic fin was female, the right male with a well-developed clasper. An ovary and both oviducts were present. On the right side there was a testis, with normal male ducts on this side only.

Miss Ruth C. Bamber described (1918) a hermaphroditic shark, *Scythum cavicula*, in which both testes were present. The anterior end of the right testis had ovarian tissue. Normal oviducts were present and the male ducts were well developed. Externally this animal was typically a male with well-developed claspers.

Most of the bony fishes have separate sexes, but certain species (*Serranus*) are true hermaphrodites. (See Shattuck and Seligmann.) Other species give exceptional individuals that have traces of both sexes. Chidester has described a male fundulus with ova attached to the mesentery of the intestine and liver.

GYNANDROMORPHS IN AMPHIBIA.

The sharp separation into adult males and females is characteristic of the group Amphibia. According to Miss Stevens there is a pair of XY chromosomes in the male of one of the urodeles, but in a frog, *Rana pipiens*, Swingle states there is only one sex chromosome in the male. Certain species of frogs pass through a stage that appears to be hermaphroditic—at least individuals that later become males may contain in the young tadpole stage large cells that appear to be incipient ova, which later disappear when the spermatozoa are formed. In the adult toad there is a region anterior to the testis proper called Bidder's organ, in which ova-like cells are present. There are a number of observations in the older literature to the effect that well-fed tadpoles produce more females than males, and *vice versa*, that starved tadpoles give an excess of males. On the other hand, there are other later observations that flatly contradict these conclusions. There are some observations, especially those of King, that show the proportion of males and of females may be determined by treating the eggs (or even the sperm) with certain substances in solution, but whether the change is due to the chemicals injuring one kind of sperm (or of egg) more than the other kind, or whether the change is of a kind to really determine the sex, irrespective of the combinations formed by the germ-cells, is open to debate. The most remarkable observations on Amphibia are those of Richard Hertwig and his pupils, particularly Kuschakowitsch. They show that by delaying the fertilization of the egg there is caused an increase in the number of

males produced. By prolonging the time to the point where the eggs are almost ready to die, all or almost all of the frogs become males. The result, moreover, appears from Kuschakowitsch's results not to be due to selective mortality. Hertwig attempts to explain the results in accordance with his view of nuclear size *versus* cell size, but the case seems peculiarly ill suited to this interpretation, because the nucleus has dissolved and the chromosomes are already in the metaphase condition when the eggs enter the oviduct, and it is here that the delay occurs. It is not at all obvious how delay in this condition can have much to do with cell size *versus* nuclear size. One of us (Morgan, 1913) has suggested that Hertwig's results may be due to a sort of parthenogenetic development in those eggs whose progress is held back. Such a result might be due either to the egg nucleus giving rise to the embryo (the sperm merely starting it, but taking no further part in the development), or to the sperm nucleus becoming the functional one, the egg nucleus having disintegrated in the interval. In support of such a view may be cited the observation of Oscar Hertwig and of Gunther and Paula Hertwig on frogs' eggs treated with radium. They interpret certain of their results as due to mononuclear development of the treated egg or sperm. The sex of the resulting larvæ was not determined. The recent results of Loeb and Bancroft and of Loeb have shown that frogs' eggs, incited to development by Bataillon's puncture method, give rise to males and females in a few cases in which the frog stage was reached. Loeb states (1918) that the parthenogenetic males have the double number of chromosomes. Herlandt describes the parthenogenetic embryos of the frog as arising in such a way that the haploid number of chromosomes at the first division must be supposed to be present, but Brachet states that he has found the diploid number of chromosomes present. Until further cytological work is done the explanation of the facts remains obscure.

Swingle has recently described hermaphroditic stages of the young frog *Rana pipiens*. Both eggs and sperm are formed in the gonad of some individuals, whereas other individuals have only testes or ovaries, *i. e.*, not mixed. He suggests the possibility that an irregular distribution of the sex chromosomes in early oögonial divisions may account for this condition. In one hermaphroditic individual he found 13 chromosomes in the spermatocytes, one of which is dumbbell-shaped, and this he thinks is the sex chromosome. In most first spermatocyte divisions the 12 autosomes divide, but the dumbbell-shaped chromosome goes to one pole. Exceptionally, however, the chromosome divides, one half going to each pole. An irregular division of the kind (or of some other kind), if it occurred at an earlier stage, might give the chromosomal combination that would produce an egg, even in a potential male.

Among the urodeles, la Valette St. George has described a newt having external male characters and an ovotestis on each side. Among the Anura several cases of hermaphroditism beside those referred to above have been described. Loisel described a frog with the secondary sexual characters of the male. On the right side no gonad was present and on the left the ovary was small and pigmented. It had no ova. This condition suggests that the male character had developed as a result of natural castration, but on the other hand, the two conditions may have had some common cause. Other cases of hermaphroditism in frogs and toads are reported by Spengel, Knappe, Hoffman, and Stephan.

GYNANDROMORPHS IN REPTILES.

Only two cases are known to me in this group—one a lizard and the other a turtle. Jacquet has described an individual (*Lacerta agilis*) that was externally a male, but had on each side a well-developed oviduct that was attached to the cloaca at one end and opened into the body-cavity at the other. No ovaries were present, however.

Fantham has described a turtle (*Testudo græca*) that had the external characters of a male. The concavity of the plastron was less marked than in a normal male. It had on the left side an ovotestis, and on the right a testis. Two ova were present in the former. Such a condition might, as suggested above for the Crustacea, be imagined to be due to chromosomal elimination, but the effect here was not localized, but extended beyond the ovotestis, since both sets of ducts were present.

GYNANDROMORPHS IN BIRDS.

The division into males and females is sharply drawn in the groups of birds, although in some families, as in the pigeons, the external differences (the secondary sexual differences) may be slight, while in other groups, owing to the development of secondary sexual characters, the external differences are very striking. In still other forms the secondary sexual characters appear only at certain seasons of the year and disappear largely at other seasons. The five cases of bilateral gynandromorphs that have been recorded make the group of particular interest in the present connection, while the exceptional conditions shown by certain hybrid crosses of pheasants call for careful analysis, especially in connection with what appears to be at least an analogous condition in hybrids of the gipsy moth.

The genetic evidence shows very explicitly that the female is heterogametic, the male homogametic. The sex-linked inheritance shown by poultry and canaries is strictly comparable to that in *Drosophila*, except that in the birds the male has two Z chromosomes (or ZZ) and the female one Z (and possibly also a W, *i. e.*, she is ZW). The cyto-

logical evidence that can be adduced in support of this view is not definitely established.

Guyer's account of the ripening of the sperm and eggs in the fowl is as follows: In the male there are 18 chromosomes, including two Z chromosomes. After synapsis there are 9 double chromosomes in the first spermatocytes, all of which except the double Z divide (or separate), 9 going to one pole, 8 to the other. Thus one daughter cell gets both Z's. This cell divides again, the Z's presumably separating, so that two second spermatocytes are produced, each with 9 chromosomes (including the Z). These become the functional sperm. The other daughter cell (without the Z's) may divide again, but it, or its products, degenerate.

In the female there are 17 chromosomes, including one Z. Presumably after reduction half of the eggs contain a Z and half are without it. The Z-bearing egg fertilized by any sperm (each carries one Z) will make a male with 18 chromosomes, including two Z's; the egg without Z fertilized by any sperm makes a female with 17 chromosomes, including one Z. The scheme will account for the sex-linked inheritance shown by the fowl. All genes carried by the two sex chromosomes of the father will be transmitted to, and shown by, his daughters, because each daughter gets her single sex chromosome from her father. If the male carries dominant genes in his sex chromosomes, both daughters and sons will show the corresponding dominant characters, etc.

It is important to observe here that while this mechanism gives the same results as to sex and sex-linked inheritance as the mechanism described by Seiler for moths, the actual process by which the two end-results are reached are quite different in the male, although presumably the same in the female. In the moth the reduction has been worked out both in the male and female, while in the bird only in the male.

Five cases of gynandromorph have been described in birds, four of which were bilaterally halved.[1] Poll described a bullfinch that had a testis on the right side, and this side had the red color on the breast characteristic of the normal male; on the left side there was an ovary, and the left side of the breast was gray like the normal female. (See frontispiece in Doncaster's book on The Determination of Sex.)

Weber gives a full account of a finch, *Fringilla cœlebs*, that had the adult male plumage on the right side and that of the female on the left side. The left side contained an ovary, the right a testis. Weber states that Cabanis (Journ. für Ornithologie, XXII, 1874) describes a "Dompfaffen" (*Pyrrhula vulgaris*) that was a bilateral gynandromorph—on the right side male, on the left female. The bird

[1] Several mixed cases in hybrid pheasants and in *Tetrao testrix* have been omitted here, as well as references to "hermaphroditic" fowls.

was not dissected. He records, apparently also on the authority of Cabanis, another bilateral gynandromorph in the species *Colaptes mexicanus*. Here, curiously enough, the right half was female, the left male, but Weber suggests that possibly the bird had the adult male plumage on the left side, while on the right the plumage was juvenile; in other words, the bird was a male, but with the full plumage only on one side, and that the left side, which normally contains the ovary.

Brandt states that Lorenz found in the markets of Moscow, in the course of fifteen years, three male *Tetrao tetrix* with female plumage; one of these had a testis on one side and an ovary on the other.

Bond has described a pheasant with the plumage of the left side preponderantly male, that of the right side preponderantly female. On the left side there was an ovary, and this is the normal position of the ovary in birds. It contained both ovarian and testicular tissue. There was no trace of a gonad on the right side. In the last three cases there is no stated correspondence between the external and the internal division of the sexes.

Setting aside the two rather doubtful cases (that of Cabanis and the uncertain reference to Lorenz's case), there remain the two well-established cases of Poll and Weber, where dissection was made, and Bond's case, that is like the last, but not so clear, since the ovary contained also testicular tissue.

It is very difficult to explain these cases by chromosomal elimination, even if the male and female plumage differences were supposed to be due to two or one (Z) chromosomes in the parts affected. Starting as a male with two Z chromosomes, if one were lost at an early division one half of the bird would be female, Z, and the other male, ZZ. This possibility could be established only by finding a bilateral gynandromorph in a hybrid that was heterozygous for sex-linked factors. Such factors have been described for pigeons (Cole and Staples-Browne) and for doves (Strong, R. M., and Riddle), for canaries, and for fowls, but no cases of gynandromorphs in them have yet been met with in which these characters were involved.

An attempt to bring the avian results in line with the *Drosophila* runs counter to the evidence from gonadectomy, since it assumes that the differences involved are due directly to the chromosomal composition of the male and the female parts, and are not due to ovarian extract, which, in poultry and ducks at least, has been shown to suppress in the female her potentiality of developing the full cock plumage. It may be interesting to review briefly this situation, since Goodale's results with ducks show that the relation of the plumage to the gonad is not so simple as appeared at first.

It has long been known in poultry that the removal of the testes does not interfere with the development of the secondary sexual plumage of the cock. In color, shape, and size of the feathers the capon

is very similar to the normal cock. The comb and wattles, however, are greatly reduced in size and have a pale color, being relatively deficient in blood. The influence of castration on the spurs is not clear, for they may be well developed in the capon and even in the hen. The influence of the ovary on the plumage of the hen has long been suspected to be important. Old hens in which the ovary had ceased to function were known to develop cock feathering, and the same result was said to follow if the ovary became diseased. But much uncertainty existed in regard to this evidence until Goodale, by carefully planned and thorough work, showed that when the ovary was removed from young birds they developed the complete plumage of the male. In the race of Leghorns the cock is red with plumage like that of the wild *Gallus bankiva*; the hens are brown. After spaying, the hens develop the complete male plumage. The spurs develop more fully than in the normal female of the Leghorn race.

When pieces of the ovary of a Leghorn hen were inserted in the body-cavity of a Leghorn capon, the latter developed only the female plumage.

In domesticated ducks (Rouen and Mallard) there are two molts. The drake molts in June and assumes his summer plumage, which is more like that of the female than is his other so-called nuptial plumage. The nuptial plumage develops during the autumn molt. If the testes are completely removed after the autumn molt the male retains his nuptial plumage even through the summer molt. Goodale finds that in normal birds, when the summer plumage reaches its highest stage of development, sexual activity diminishes or disappears, and few or no sperms are present. It is at this time then that the drake develops his nuptial plumage, as removal of feathers shows. In other words, it is the summer plumage (the one that is more like the female) that develops when the sexual organs are at the highest development, while the nuptial plumage develops when the sperms are not being produced in the testes. It appears, then, that the nuptial plumage is not influenced by the testicular condition, while the female-like plumage may possibly be due to the inhibitory effects of the testicular secretions. In other words, the case is somewhat like that of the Sebright, in which the presence of the active testis suppresses the potential cock feathering of the male.

These results do not appear to furnish any solution of the problem of bilateral gynandromorphs in birds, because the chief difficulty remains so long as any internal secretion, whether ovarian or testicular, determines in an individual the character of its plumage. Any theory of bilateral gynandromorphs in birds must be prepared to offer some explanation as to why the ovarian extracts do not suppress in them the male feathering on the male side. Two more or less plausible answers can be given at present. One of them is that in certain

species of birds the male plumage is not affected by ovarian secretions, as it is in poultry and in ducks, but is due directly to genetic factors that act effectively in the male but not in the female. It ought to be comparatively easy to find this out for each race by means of gonodectomy.

The other possible explanation is that although in a bird genetically male (ZZ) on one side and female (Z) on the other, the secondary sexual characters would be female; yet if the ovary should become diseased or old and its secretions diminished, a point might be reached where the secretion could no longer hold in check the full development of the male part. The bilateral gynandromorph in birds would on this view represent only a transient stage. In point of fact, none of them have been kept alive for any length of time, so that we do not know that they would hold their superficial peculiarity. An alternative to this view that the secretions were insufficient because of disease or age is to suppose that the ovary is abnormally small from accident or heredity. In this case the gynandromorph stage would be more permanent. Such birds would be expected in all cases to have an ovary, or at least to have some traces of one, unless the species resembled Mallards or Sebrights, where the testis influences the plumage.

The results that Riddle has reported concerning intersexes in hybrid pigeons do not call for detailed review here, since the phenomena recorded relate largely to behavior. Riddle believes that under "conditions of overwork" a female produces eggs, some of which are male-producing, others female-producing, as shown by mating such females to the males of their own species when equal numbers of males and females are produced. But such overworked eggs, if fertilized by a male of a different genus, produce predominantly female birds. The result, however, is not attributed to the male, or to the cross, but to some change in the egg that causes a reversal of the sex tendency.

The only case that Riddle has reported in which the color inheritance is given, so that one can follow the sex-linked heredity in connection with the abnormal sex ratio, is that recorded in the *Naturalist* for 1916.[1] The first 17 doves were 5 male to 12 female doves; the second 17 doves were 4 males to 13 females; the last 17 doves were 2 males to 15 females. The cross was made between *Streptopelia alba* male and *St. risoria* female. As R. M. Strong had previously shown, the expectation here is for dark sons and white daughters. Since the reciprocal cross gives all dark offspring, the factor involved is sex-linked and not merely sex-limited. Riddle obtained only dark males and white females, except two that were dark (one being questioned by himself). Strong also found a few dark exceptions, as did also Staples Brown. As Bridges has shown, these exceptions can be

[1] Reproduced and expanded in the Journal of the Washington Academy of Sciences, June 1917.

explained by non-disjunction. They are too few in any case to affect Riddle's argument based on the sex-ratio. It follows, then, that Riddle's results, instead of showing that some females started as males, show exactly the reverse, since the genetic history shows that all his females must have had the genetic chromosome constitution characteristic of the female and have gotten it in the usual way.

GYNANDROMORPHS IN MAMMALS—MAN.

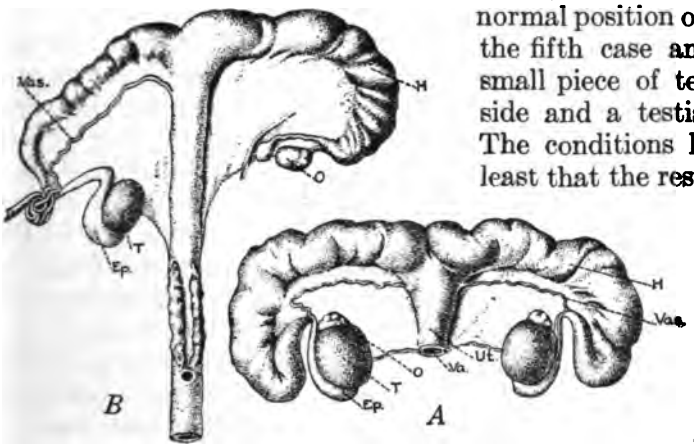
Cases of true hermaphroditism or gynandromorphism in mammals and in man are extremely rare. From the meager evidence it is not clear whether the cases reported belong under one or the other head, but there are, as far as we know, very few if any cases of strictly bilateral gynandromorphs. The secondary sexual differences, while not so marked as in some other groups, are yet sufficient, one would suppose, to make a bilateral type clearly evident. Goldschmidt has suggested that intersexes occur in man of the kind shown by the gipsy moth. So far, at least, there is no positive evidence to show that such individuals occur more frequently in racial crosses in man than within the race, but the human races are themselves so mixed in origin that this point may not have any critical value for the subject. *A priori*, it is equally possible that the intersexual individuals, if genetic ones exist, may be due to autosomal differences that affect the normal instincts rather than to differences in the sex genes themselves. It is not claimed, I believe, that the actual sex-organs themselves are involved, but rather secondary sexual characters and instincts whose relation to the sex mechanism are in man entirely obscure.

According to Rudolphi, there is a record by Schlumpf (Arch. f. Thierheilkunde, Zurich, 1824, pp. 204–206) of a calf externally like a male, but in place of the scrotum there are present the udders with the usual number of nipples. The uterus had only one horn and funnel, and an ovary fastened to right side of "der Leiden." To one kidney (left) was attached a small testis. Rudolphi also describes a seven weeks' old child that lived about three months that had a hypospadic penis and in the right scrotum a testis, but no testis in the left. There was a uterus whose left upper end was connected with a Fallopian tube attached to which was an ovary. On the right side the uterus ended blindly and there was neither Fallopian tube nor ovary present. Two very similar cases, one by Gautier (1752) and one by Pinel, are referred to by Rudolphi.[1]

According to Pick (1914), Sauerbeck admits only 7 cases of hermaphrodites in mammals as certain and complete, 5 for swine, and 2 for man (Salens, 1899, and Simon, 1903), to which number are added 3

[1]Rudolphi (1825) refers to two supposed cases of hermaphrodites in fowls, which he very properly questions.

cases for mammals (roebuck and goat) and 5 for man as very probable. To these Pick adds a later case by Uffreduzzi (1910) and one by Gudernatsch (1911). The 5 cases found in swine by Pick are of unusual interest. The external genitalia were entirely female or nearly so. Within the abdomen were uterus and fallopian tubes (text-fig. 70). In four of the cases an ovotestis was present on each side in the normal position of the ovary. In the fifth case an ovary with a small piece of testis was on one side and a testis on the other. The conditions here suggest at least that the results are not due to chromosomal elimination, although such an interpretation might be given. If, for instance, the gonads arise at an early stage from a single cell in which an anterio-posterior division occurred and the later mass of cells was subsequently separated into right and left parts, the conditions found might be realized. There is, however, a possibility that here, as in cattle, a union between the chorions of the embryo in the uterus might have brought about a more perfect freemartin than develops in cattle when such a union occurs. (See Lillie.)



TEXT-FIGURE 70.

Ritter described a pig in which on the right side a testis was present and on the left an ovary. (Verh. phys. Med. Gesell. Würzburg, XIX, 1886). Harman more recently (1917) describes a cat with a testis on one side and an ovary on the other. Neurgebauer has given a large number of cases in man in which testes and ovaries have been described in the same individual and in which the genitalia show many anomalous relations. Amongst the large number of human hermaphrodites described there are probably a considerable number of authentic cases where parts of both male and female genitalia were combined in the same individual, but writing as late as 1911, Gudernatsch states that hermaphroditism in the sense of separate ovaries and testes has not been demonstrated in man. He describes a case of an individual with female external genitalia and an abnormal testis in the right inguinal canal.

The proof that hermaphroditism, so-called, in man is produced in the same way as gynandromorphism in *Drosophila* can not be furnished at present, because there is no probability of the difference in chromosome number being determined by histological study, owing to the

large number of chromosomes, nor is it probable that a case involving sex-linked factors will soon be found.

Some of the older writers seem to mean by hermaphroditism the presence of complete sets of both male and female organs, the two systems superimposed on each other. The rather mythical accounts of such cases do not call for serious comment.[1] Where the evidence is anatomical and given by trained observers it appears that some, perhaps all, cases are mosaics rather than "hermaphrodites" in the sense of double-sexual individuals. In other words, parts of one and parts of another system are found in the same individual, replacing each other locally. If this interpretation turns out to cover certain cases the theory of chromosomal elimination will suffice at least as a formal explanation of such human abnormalities. Since the human species is both from the genetic and cytological evidence XX in the female and XO in the male, the same mechanism exists as is found in *Drosophila*, and if the theory of chromosomal elimination applies here also, human gynandromorphs would be expected in practically all cases to begin as female (XX) and produce male regions by eliminating one X. An examination of the literature shows in fact a considerable preponderance of the cases showing more female than male regions, but the evidence is too uncertain to give any serious weight to it.

IS CANCER A SOMATIC MOSAIC?

Into the difficult and obscure question as to the cause of cancer it is not our business to enter, but a suggestion made by Boveri (in 1902 and 1914) calls for brief notice, since he appealed to a process akin to chromosome elimination as a possible explanation of the phenomenon. Boveri suggested that an imperfect or irregular division of the chromosomal complex might in certain cases produce combinations through loss of specific chromosomes that caused the different cells to run wild, so to speak, in the sense that factors that normally inhibit the rate of growth or the suppression of growth in relation to the cell environment are lost. In support of such a view he appealed to occupational cancer-growth, where cancer develops in parts of the body most subject to mechanical injury or pressure. It is known to students of embryology that compression of a dividing cell may interfere with the normal distribution of the chromosomes to the daughter cells. At present, however, reference to such possible sources is too uncertain to be of great value, for there are no instances where irregularities of this kind are known to give rise to prolific growth processes. The cancer-like or tumor-like growth shown by a mutant race of *Drosophila*, discovered by Bridges and described fully by Stark, has not been shown to be associated with abnormal distribution of the

[1] See comment by Dr. H. L. Garrigues, Medical Record, 1896, p. 725.

chromosomes, although this point has not been sufficiently studied to exclude such a process. On the other hand, it has been shown that the growth in question is caused by a sex-linked Mendelian gene that is inherited strictly, as are all Mendelian sex-linked genes. This mutant lethal race of *Drosophila* arose as a mutation, presumably in the same way as other mutations. If it is not admissible at present to draw any analogy between this case and that of mammalian cancer, it is conceivable at least that mammalian cancer may be due to recurrent somatic mutation of some gene. Such a conclusion would, however, not invalidate the view that cancer is more likely to occur in certain families, or even be inevitable in them, because recurrent mutation in certain genes appears to be more likely than in other genes. But even if this view were maintained the inheritance would be different in kind from the inheritance of ordinary Mendelian genes, because such a view involves a secondary step, viz, the likelihood of a mutation in a race containing the inherited gene in question. The whole problem of the causes of mutation is at present so obscure that a discussion of this possibility is purely theoretical. Added to this is the uncertainty of how cancer is inherited in those races of mice that appear to produce it with great frequency. Important as the work along these lines unquestionably is, the subject is not yet ripe for any positive statement. It may, nevertheless, be worth while to keep in view the possibility suggested above, viz, that what is inherited in cancer may be a gene or complex of genes in which somatic mutation is of sufficient frequency to give the appearance that a gene for cancer is itself inheritable.

IS THE FREEMARTIN A GYNANDROMORPH?

It has been suggested that the pair of twin calves, one male, the other a sterile female (the freemartin), together represent a sort of gynandromorph. This view is based on the assumption (which Lillie has since disproven) that these twins arise from a single egg. Hart (Proceedings Roy. Soc. Edinburg, XXX, p. 218) suggested that "the freemartin with a potent bull twin is the result of a division of a male zygote, so that the somatic determinants are equally divided and the genital determinants unequally divided, the potent going to one twin, the potent bull, the non-potent, genital determinant to the freemartin." It is needless to point out that this vague statement can not be brought into accord with embryological evidence, because Lillie's work shows that each individual of the twins arises from a separate egg. In most cases the eggs arise from the two ovaries, and each embryo lies in a different horn of the uterus.

Lillie has shown that in those cases where twins are present, one of which is a freemartin, the two chorions and the two allantois have fused at an early stage, and he has demonstrated that there is an

actual vascular connection between the two individuals. There can remain no doubt that the results are due to the establishment of a common circulation. Lillie brings very strong evidence in favor of the view that the freemartin starts as a normal female. The failure of her ovary to develop, he thinks, is due to a sex hormone (see below) that originates in the testis of the male and suppresses the normal development of the ovary. The external genitalia of the freemartin, and to some extent the uterus and ducts, are, as a rule, less affected by the hormone, so that externally the freemartin appears to be a female. Even more remarkable is the fact that the male ducts are sometimes quite well developed and the development of the ovary appears to take in somewhat the characteristic changes seen in the development of the testis. This conclusion is based largely on the results of a histological examination by Miss C. L. Chapin. Lillie is not inclined, however, to lay very much emphasis on this side of the question, because, as he states, the suppression of the ova (and female stroma?) may in itself allow some of the male characteristics to develop to a stage not normally present in the female. In other words, the development of the accessory organs may to a certain extent be under the influence of the gonad.

The assumption of a male hormone originating in the interstitial cells of the testis is more problematical. The only fact advanced by Lillie in favor of this interpretation is that in the testis the interstitial tissue develops at an earlier stage than that in the ovary. It is true that there is also some evidence indicating that the interstitial cells of the testis produce some substance that affects the secondary sexual characters of the male. But it may be that other substances in the blood of the male affect the ovary of the freemartin and retard its development. Such substances might also be called hormones, but have no direct relation either to the development of the germ-cells in the testes or to *sex determination in any specific sense*. If in cattle the male differs from the female by one sex chromosome, it is quite possible that the composition of the blood of the male is different in some substances (or relative proportion of substances) from the blood of the female. The difference, while the product of sex in the sense that all the body-cells of the male differ by one chromosome from the body-cells of the females, might not in any way be connected with sex determination, even although it affected injuriously the development of the ovary of the young female embryo. Until further evidence is obtained, the source of the "hormone" that affects the freemartin must remain an open question.

If the ovary of the freemartin is actually changed to a testis it may be said that the freemartin is a sex mosaic, the external genitalia female and the gonads more or less male. The cause of such a sex mosaic would, then, obviously, be entirely different from the cause of the gynandromorphs of *Drosophila*.

SUMMARY.

(1) (*a*) The main outcome of this work on gynandromorphs of *Drosophila* is an experimental demonstration of the principal cause of the regional differences that gives rise to the combinations of male and female in the same individual. The demonstration was made possible by taking advantage of the genetic situation in this material.

(*b*) Many of the gynandromorphs were hybrids of known sex-linked characters, *i. e.*, characters whose genes are carried by the sex chromosomes.

(*c*) By adding to such crosses additional characters whose genes lie in other than the sex chromosomes it has been possible to prove that the male and female parts of the gynandromorph differ by the sex chromosomes alone, *i. e.*, both male and female parts contain the same autosomal group.

(*d*) It was possible, in consequence, to show that these gynandromorphs are not due to partial fertilization (Boveri), or to polyspermy (Morgan), but to chromosomal elimination (Morgan). Chromosomal elimination means that at an early stage in embryonic development one of the daughter chromosomes of one of the X's fails to pass over to one of the daughter plates, and accordingly gets left out of that nucleus. In consequence, one of the two cells will contain only one X chromosome and produce male parts, while the sister cell with two daughter X chromosomes will produce female parts. The evidence that elimination of this kind takes place rests on cases in which the X chromosome derived from the father contains different sex-linked genes from the X chromosome derived from the mother.

(*e*) A census of the available gynandromorphs shows that a paternal X chromosome is eliminated as often as a maternal X chromosome.

(2) A logical consequence of the proof that the gynandromorphs arise through elimination is that they should all start as females, *i. e.*, as XX individuals. If the elimination always takes place at the first division the expectation would be for the male and female parts to be equal; but if at the second, third, or any later division of the nuclei, we should expect to find, on the whole, a preponderance of female parts over male parts. Such is strikingly the case.

(3) A second logical consequence of chromosomal elimination is that starting as an XX individual; the male parts will be XO, and not XY as in the normal male. Now, it has been shown by Bridges (1916) that XO males arising from primary non-disjunction are sterile (although in structure, etc., they are exactly like XY or normal males). The great majority of gynandromorph individuals with male abdomen and testes are infertile, while if the corresponding parts are female the individual is fertile. The few gynandromorphs, fertile as males, are known from other genetic evidence to have come from XXY

mothers or to be themselves XXY zygotes. In such cases, after elimination, the male parts are expected to be XY, and hence an individual of this origin with a male abdomen and testes is expected to be fertile.

(4) A striking fact in regard to these gynandromorphs is that the male and female parts and their sex-linked characters are strictly self-determining, each developing according to its own constitution. No matter how large or how small a region may be, it is not interfered with by the aspirations of its neighbors, nor is it overruled by the action of the gonad.

(5) Four experiments were made in which suitable material was carefully scrutinized for gynandromorphs. In the 88,000 flies, there were found 40 gynandromorphs, or 1 to 2,200. Since only those that start as females give this kind of gynandromorph, chromosomal elimination may have occurred once in 1,100 individuals.

(6) (*a*) If chromosomal elimination took place at the first division of the segmentation nucleus, a half-and-half gynandromorph is expected (right-and-left or anterio-posterior). Whether dorso-ventral separation is expected for such a division depends on whence comes the material that ultimately reaches the dorsal surface of the fly.

(*b*) If the chromosomal elimination took place at the second-division period in one of the nuclei only a quadrant is expected to be male, etc.

(*c*) The fact that most of our mosaics include large regions of the body may mean that elimination takes place more often during the first or second division, but it may also mean that when smaller regions are involved the gynandromorph would be more often overlooked.

(7) (*a*) Both gonads of the same individual are always alike, *i. e.*, both are testes or both are ovaries, even when the external markings of the abdomen are male on one side, female on the other. This result finds its explanation in the assumption that the germ-plasm of *Drosophila*, as in some other flies, arises from a single cell. This cell, arising after elimination, must be either a spermatogonium or oögonium. If the cell be the former the sex-linked factor of the germ-plasm must be that of the male-determining X chromosome alone and not show any of the factors contained in the other X of the female parts. Such is the case.

(*b*) Conversely, the ovary of a gynandromorph containing both X chromosomes should produce eggs containing the original X chromosomal combinations as well as their cross-over combinations. This, too, is the case.

(8) It is a striking fact that we have found so few cases of autosomal elimination. The lack of such mosaics may be due to the failure of the ordinary chromosome to lag in division as the X is assumed to do, or it may be that a fly or part of a fly can not exist if one autosome is absent from its complex. That a part may exist with one X chromosome lost might be explained as due to that condition having been

already acquired by the male. Future work must show whether or not such autosomal mosaics are viable.

(9) Courtship has been watched in a number of flies that were partly male and partly female. Many of them are indifferent; some react as males, some as females.

(10) In several cases flies that had one white eye and one red eye have been observed to show circus movements. Since the white-eyed fly is less responsive to light than the red-eye fly, the circus movements of the gynandromorph with one white and one red eye is what is to be expected. Of course, such cases must be selected so that the legs are not male on one side and female on the other.

(11) The general evidence from mutation in *Drosophila* makes it highly probable that when a mutation occurs it takes place in only one chromosome of the pair. Hence any mutation in somatic tissue, if recessive, would be concealed by the presence of the normal allelomorph in the homologous chromosome. If, however, a mutation should appear in the sex chromosome of the male, even though recessive, its effects might be apparent. It is probably significant that the ten cases here described and supposed to be somatic mutations are all males.

(12) Theoretically, at least, there is the possibility that an individual starting as a male might produce female parts. If at some embryonic division both daughter X's of an XY cell should pass into the same cell, it would be expected to produce female parts. There is, however, a difficulty with the other cell containing a Y chromosome and no X. It would probably die.

(13) In addition to the two earlier theories of Boveri and Morgan mentioned above, other theories are critically considered from the point of view of the gynandromorphs of *Drosophila*. The only other theory besides elimination that we have found necessary to employ in accounting for the gynandromorphs of *Drosophila*, where the genetic evidence makes the analysis possible, is the theory of binucleated eggs.

(14) In the light of the evidence from *Drosophila*, both the Eugsterbee gynandromorph and von Engelhardt's gynandromorph can be accounted for on the hypothesis of chromosomal elimination, especially since the work of Newell and Quinn shows that the racial characters involved differ in one Mendelian gene (though not necessarily one in the sex chromosome). However, in both cases, if paternal and maternal elimination are equally likely in both combinations, as many gynandromorphs showing the racial character of only one type are expected as those mosaic for racial characters as well as for sex. Such have not been reported.

(15) (*a*) In moths several gynandromorphs have been reported that were mosaics for paternal and maternal characters well as as for sex. Some of these, starting as males, can be explained by chromosomal elimination.

(*b*) In *Abraxas* a factor involved is known to be sex-linked. Two mosaics between *A. grossulariata* and *lacticolor* described by Doncaster can be accounted for by chromosomal elimination in one case and by a non-disjunctional sperm and elimination in the other.

(*c*) The two gynandromorphs in silkworms described by Toyama can be explained, genetically, on the basis of two nuclei present in the eggs. Doncaster has found such eggs in *Abraxas*.

(*d*) Whether the mosaics in the gipsy moth, formed by racial crosses, are due to different sex-factors having different quantitative value, as maintained by Goldschmidt, or due to some other relations, seems uncertain from the evidence so far published.

(16) In reviewing the literature it is pointed out that in the Crustacea and molluscs there are several cases where an individual is male at one period of its life and female at another, just as some plants pass through similar stages. In such cases the environment, taken in the widest sense, may suppress one sex and develop the other. The influence of the environment is clearly shown in the case of the crabs infected by *Sacculina*, where the secondary sexual characters are changed; and in *Crepidula*, where proximity to another individual effects a change of sex, and in the worm *Bonellia*, where a similar change is brought about. There is nothing here that is in the least inimical to the view that in other cases, and even in these same groups, there may be genetic factors that determine sex under ordinary or other circumstances. The bilateral gynandromorph of the crayfish (p. 97) may be a case in point.

(17) A few cases of bilateral gynandromorphs in birds have been reported. Their occurrence is unexpected because of the known effect of the ovary in suppressing most of the secondary characters of the male. It is suggested that in some species of birds, particular secondary sexual differences are not influenced by internal secretions, hence a gynandromorph condition in the chromosomal composition might show itself in plumage characters. It is also suggested that if a bird showed the female complex in one region and a male complex in another the amount of internal secretion that might inhibit one side might be insufficient to inhibit the other. A transient or an abnormal condition of the ovary might make the gynandromorph differences visible.

(18) In man and in other mammals a number of cases of gynandromorphs are known, some of them at least well authenticated. Most of the cases rest on the condition of the gonads and accessory sexual organs. Sex mosaics like those of *Drosophila* are expected, because the mechanism of sex determination is the same. On the other hand, in the light of Lillie's evidence for the freemartin, other kinds of modifications may be possible. Even in cases where only a single individual is born an earlier connection with an absorbed or aborted embryo might be responsible for an abnormal condition of the sexual organs.

POSTSCRIPT.

Professor F. R. Lillie has called my attention to two important papers on freemartins. It appears that Tandler and Keller[1] had already published, in 1911, the essential facts relating to the vascular connection between the embryos *in utero*, leading to the development of the freemartin out of the female member of the united pair. They had also shown that the embryos come from two eggs. Magnussen[2] in 1918 has described a considerable number of cases of freemartins. He regards both individuals as having started as males, and compares the usual rudimentary condition of the testes of the freemartin with that of a cryptorchid testis. He adduces no evidence of importance in favor of his view that the twins started as males, while Lillie's evidence is convincing in support of the view that the freemartin started as a female.

The most important facts reported by Magnussen are those relating to the histological condition of the testes of the freemartin. Well-developed testes are present in some of the older freemartins, ranging in size from that of a hazel-nut to that of a hen's egg. The vasa deferentia, the epididymus, and notably even the tubular tissue characteristic of the testes were present, but no germ-cells were found. Now the absence of germ-cells from the tubular tissue of the testes of the adult freemartin may be accounted for, as Magnussen does account for it, viz, as due to the "retention" of the testes of the freemartin. This condition would not, however, be expected to hold for the embryonic testes, where in the walls of the testes at birth one would expect to find the germ-cells present. If a critical examination of these stages shows that germ-cells are not present in the tubules of the testes of the freemartin, then the evidence from the freemartin shows not that the sex of the female has been changed, but that under the influence of the blood of the male the accessory organs, as well as the secondary sexual organs characteristic of the male, have developed in the female; while at the same time her own female accessory organs have correspondingly failed to develop fully. This statement implies that the critical evidence for sex is the kind of germ-cell produced, while the development of the secondary sexual characters and of the accessory organs of reproduction in the mammal is determined, in part at least, by the germ-cells. It will be recalled that, according to the most recent work in mammalian embryology, the germ-cells originate in or from the region of the intestinal tract, far removed from the final position in the gonad into which they find their way by migration. If, then, it prove that no true germ-cells are found in the testicular tubules of the freemartin, the presence of the "testes," including even the epididymus, and tubules demonstrates only how far the origin of these parts is dependent on something in the male; but whether this something comes from the germ-cells of the male (directly or indirectly) or is a consequence of the genetic composition of the male is not shown.

July 17, 1919.

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[1] Tandler und Keller, 1911. Ueber das Verhalten des Chorions bei verschieden-geschlechtlicher Zwillingsgravität des Rindes, uhd ueber die Morphologie des Genitales der weiblichen Tiere, welche einer solchen Gravität enstammen. Deutsche tieraersliche Wochenschrift. (No. 10.)

[2] Magnussen, H., 1918. Geschlechtslose Zwillinge. Eine gewöhnlich Form von Hermaphroditismus beim Rinde. Archiv. f. Anat. u. Physiol. Anat. Abt.

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II.

THE SECOND-CHROMOSOME GROUP OF MUTANT CHARACTERS.

By C. B. Bridges and T. H. Morgan.

With seven plates and seventeen text-figures.

THE SECOND-CHROMOSOME GROUP OF MUTANT CHARACTERS.

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INTRODUCTION.

This paper deals with 39 mutant races whose genes lie in the "second chromosome." This number includes all of those previously described, and gives a complete account of all the second-chromosome mutants found before 1916. The latter have been for the most part used or mentioned in previous papers by ourselves and others. It has been our rule never to hold back any useful mutant type until it had been recorded by its discoverers, and in consequence a number of the characters here described for the first time have already been used widely. This applies equally to the third-chromosome mutants, an account of which we hope to publish soon.

In addition to the 39 mutant types here described there remain about 35 others discovered since 1915 which are still to be described. In making our selection for the present publication we have included those which are most essential for future work both in localization of genes and in special experiments. For example, star eye, because of the location of the gene at the extreme 'left' end of the chromosome, of its dominance, and of its other excellent characteristics, is now the most generally useful second-chromosome mutant. Again, purple eye-color is the only workable eye-color in this chromosome. It has been involved in the localization of many of the genes in the chromosome. Its position is at the center of the chromosome, which center shows certain important peculiarities. Furthermore, its location near black gives a working distance suitable for analyzing linkage and coincidence characteristics; the distance between purple and black is short enough to exclude double crossing-over and long enough to exclude the large and uncertain probable error incident to small percentages of crossing-over. A third important mutant type, speck, whose gene is located near the right end of the chromosome, is used as the basis of reference of the genes of that end of the chromosome. Finally, curved is, in addition to its excellent viability, ease of identification, and other useful features, very valuable from its position at the right of the central group of the most useful and best located genes.

The mutants have been discussed in order of their discovery, since this method involves least use of material requiring special explana-

tion, and the earlier experiments were relatively simple. In the case of several of the mutants found very early, little more than a review of the extensive work already published has been given.

A chronologically arranged list of all these mutants, together with a summary of main points with respect to their origin, locus, etc., is given in table 1.

TABLE 1.—*Chronologically arranged list of II-chromosome mutant genes treated in this paper.*

Mutant gene.	Affects mainly—	Fig.	Symbol.	Locus.		Date found.	Arose from—	Culture No.	Found by—
				$S'+$	Primary base.				
Speck	Axil of wing	Pl 5, figs. 1 and 4, text-fig. 73.	*sp*	105.1	*c*+31.6	1910 Mar. —	Wild stock		Morgan.
Olive	Body-color	Pl. 5, fig. 1.,	*ol*	106.1 =	*sp*+ 1. =	May —	Do.		Do.
Truncate	Wing-length	Pl. 6	*T'*	28.0	*S'*+28.0	Aug. —	Beaded stock		Do.
Black	Body-color	Pl. 5, fig. 2	*b*	46.5		Oct. —	Miniature experiment.		Do.
Balloon	Wing, venation	Pl. 7, fig. 1	*ba*	105.5	*sp*+0.4	Nov. —	Truncate stock		Do.
Vestigial	Wing, balancer	Pl. 7, fig. 2	*vg*	65.0	*pr*+12.3	Dec. —	Do.		Do.
Lethal *T'*	Life		*l T*		*T'* =15	1911 Feb. —	Do.		Do.
Blistered	Venation	Text-fig. 74.	*bs*	103 =	*sp* − 2 =	Nov. 16	Rudimentary stock.	A 23	Bridges.
Jaunty	Wing curvature	Pl. 7, fig. 3	*j*	46.7	*b*+0.2	Dec. 11	Do.	A 34	Do.
Curved	Do.	Text-fig. 75	*c*	73.5	*pr*+20.8	Dec. 24	Do.	A 43	Do.
Purple	Eye-color	Pl. 5, fig. 8	*pr*	52.7	*b*+ 6.2	1912 Feb. 20	*vg* stock	A 66	Do.
Strap	Wing	Pl. 8	*vg*^{s}	65.0	*vg* = 0.0	Apr. —	Do.		Morgan.
Arc	Wing curvature	Pl. 7, fig. 4	*a*	98.4	*sp* −6.7	May 24	Black-palpi stock.	B 30	Bridges.
Gap	Venation	Text-fig. 76	*gp*			July 10	*b*×*a*	B 42	Do.
Antlered	Wing	Pl. 9	*vg*^{a}	65.0	*vg* = 0.0	Sept. —	*vg* experiment		Morgan.
Dachs	Venation	Pl. 10	*d*	29.0	*b* −18.5	Oct. —			Do.
	Legs	Figs. 1*c*–1*d*				Nov. 22	Sable experiment.	C 146	Bridges.
Streak	Thorax pattern	Pl. 5, fig. 5. Pl. 10, fig. 2.	*Sk*	15.4	*d* −13.6	Nov. 27	'Lop' stock	C 149	Do.
Comma	Thorax marks	Text-fig. 79			*Sq* = 15	1913 Feb. 5	*d*×*pink*	II 9	Do.
Morula	Eye-facets	Pl. 10, fig. 3	*mr*	106.3	*a*+ 7.9	Mar. 8	*peach*×wild	M 20	Do.
Apterous	Wing, balancer	Pl. 7, fig. 5	*ap*	48.5	*S'*+48.5 =	Aug. —	*ws* stock		Wallace.
Cn I	II crossing-over		*Cn II*		*b* −?	Sept. —	"Nova Scotia"	7	Sturtevant.
Cn	Do.		*Cn*		*px* −?	Sept. —	Do.	7	Do.
Cream II	Eye-color	Pl. 5, fig. 10	*cr II*			Sept. 15	Lethal 2 stock		Bridges.
Patched	Abdomen	Pl. 11				Nov. 25	*cr II*	M 68	Do.
Trefoil	Thorax pattern	Pl. 5, fig. 6. Pl. 8, fig. 1.	*tf*	50 =		Nov. —			Morgan.
Cream *b*	Eye-color	Pl. 5, fig. 11.	*cr b*	22.5		1914 Mar. 10	Non-disjunction.	82	Bridges.
Pinkish	Do.	Pl. 5, fig. 12.		100. +	*b*+60 =	July 27	Eosin *b* stock		Do.
Plexus	Venation	Text-fig. 80	*px*	96.2	*sp* − 8.9	Aug. 20	Spread stock	557	Do.
Limited	Abdomen	Text-fig. 81		106.3	*mr*+?	Sept. 13	*ws* stock	511	Do.
Confluent	Venation		*Cf*			Sept. 23	Non-disjunction.	550	Do.
Fringed	Wing	Text-fig. 82	*fr*	98.0 =	*b*+51.5	1915 Jan. 20	Jaunty I	1,042	Do.
Star	Eye-facets	Text-fig. 83	*S'*	0.0	*Sk* −15.4	Feb. 12	Non-disjunction.	1,347	Do.
Nick	Wing	Text-fig. 84	*vg*^{n}	65.0	*vg* = 0.0	May 7	Lethal 2	2,012	Do.
Dachs-lethal	Life		*d l*	29.0	*d* = 0.0	Oct. 6	*S'*×*d*	2,217	Do.
Squat	Wing	Text-fig. 85	*Sq*	35.5	*b* −11.0	Nov. 29	Non-disjunction.	2,480	Do.
Lethal IIa	Life		*l II a*	62.7	*c* −10.8	Dec. 2	*S'b c* stock	2,675	Do.
Telescope	Abdomen, wing	Pl. 7, fig. 6.	*ts*	66.5		Dec. 27	'Crooked' experiment.	2,735	Do.
Modifiers	Dichaete				*sp* −?	1916 Aug. 13	*D* stock		Sturtevant.
Dachsoid	Venation	Text-fig. 86				1917 Feb. 9	Eosin × 'sepia'	2,671	Do.

*The mutations marked with the asterisk (*) are no longer on hand, having been lost or discarded.

Special attention has been given in the treatment of the data to bring out the genetic methods employed, and to trace the development of such methods. The part each mutant has played in this development of methods and principles has been given fully, often at the expense of repetition. An effort has been made to evaluate each mutant with respect to its usefulness as a working tool. Some are practically useless, while certain others, from the possession of excellent characteristics or favorable location in the chromosome, etc., must be considered in every carefully planned experiment. By such methods of presentation it is hoped to make available not simply a body of data but also a working familiarity with the material.

From the various published and hitherto unpublished materials on crossing-over in the second-chromosome a summary is given of total data available on amount of crossing-over between various loci (table 140). The cross-over values calculated from these data are still further summarized and presented in graphic form in the map of the second chromosome which appears as fig. 72.

In the construction of this map it was necessary to correct some of these values by aid of information gained from a study of the amount of double crossing-over and coincidence in the various regions of the second chromosome. Details of how this was done will be found in the last section, which deals with the construction and use of this map. The coincidences, and consequently the corrections, are at present only quite rough approximations, and the same is true of the methods of weighting employed in that section. It should be borne in mind that the map is a composite picture in which differences in the data from different sources are no longer apparent. Reference to these separate data will show, however, a very surprising uniformity, especially in view of the many conditions now known to be able to cause the amount of crossing-over to vary.

A glance at the map shows at the right end (bottom) an unusually dense cluster of genes. When

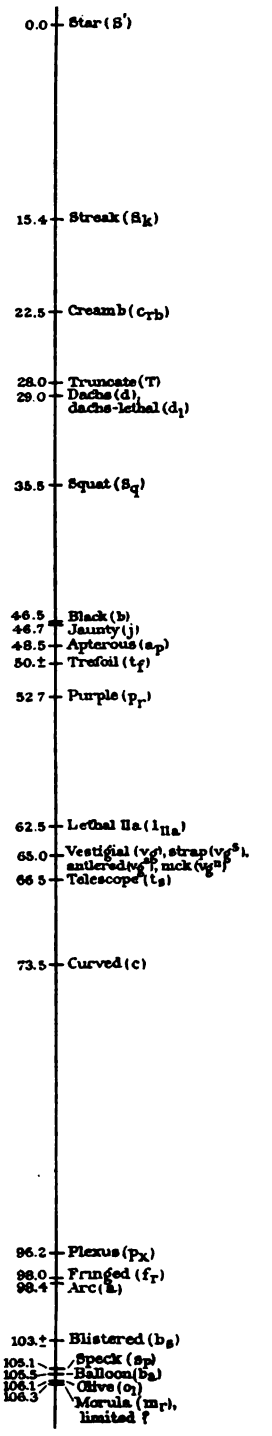


FIG. 72.

TEXT-FIGURE 72.—Map of the second chromosome, giving the locations with reference to star, and the symbols of the mutants whose loci are known.

we come to add to the map the loci of the genes as yet unpublished, another such cluster will appear at the left end of the chromosome also. These clusters at the ends may be looked upon, not as due to the genes being here actually nearer together, but to the probability that at the ends crossing-over is relatively less frequent than in the middle part of the chromosomes. The bearing of the information as to the relative frequency of double crossing-over on the conclusion just stated is discussed in the section on "Purple."

To the reader who is not especially concerned with the localization of the genes, we should like to call attention to other subjects of very general interest, such as the discussion of modifiers in the sections on purple, the creams, star, and the second-chromosome modifier of the third-chromosome character, dichæte. Another topic of general interest is that of autosomal and balanced lethals discussed in the sections on truncate, streak, confluent, star, dachs-lethal, and lethal II*a*. A third topic of interest is that of variations in the amount of crossing-over due to specific genes and to such factors as age and temperature that are discussed in the sections on purple, dachs-lethal, and in the summary dealing with the cross-over variations $C_{II\,l}$, and $C_{II\,r}$.

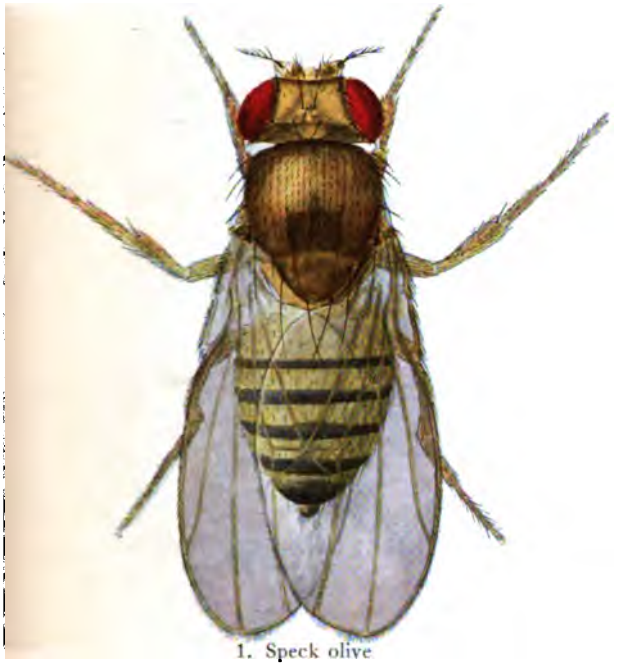
SPECK (*sp*).

(Text-figures 73 *a* and *b*, 75 *b*, and plate 5, figures 1 and 4)

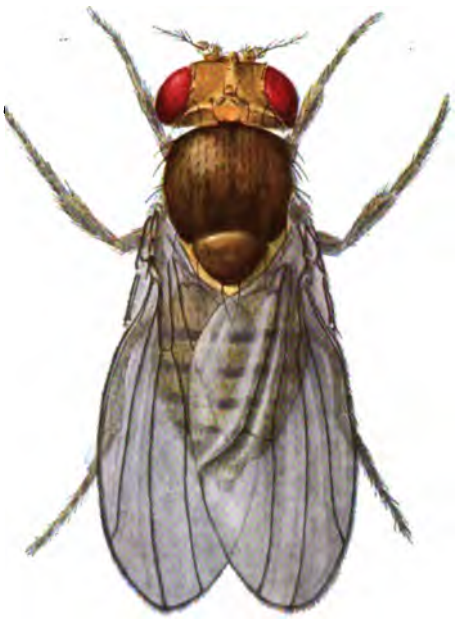
ORIGIN AND STOCK OF SPECK.

In the course of the early work upon *Drosophila* in the Columbia Zoological Laboratory a selection experiment was carried out by Morgan upon a race of wild flies that had showed variation in the extent and darkness of the shield or trident pattern upon the thorax. In the fourth generation of selection for a race "without" such a trident, there appeared (March 1910) a few individuals with a tiny black speck (plate 5, fig. 4) at the juncture of each wing with the thorax (Morgan, 1910). At first the breeding results obtained with this character were irregular (Morgan, 1910). Some of this irregularity may have been due to non-virgin females (24-hour females were used) and to the practice of using mass cultures, though probably more was due to difficulty of classification before familiarity with the characteristics of the mutation had been acquired.

A stock pure for the character was obtained, but was set aside in order that more time might be given to the study of the sex-linked eye-color white which had appeared in April 1910. About a year after this (May 1911) it was found that a stock of flies with a dark body-color called "olive" was pure for a character which was taken to be the same as speck (text-figs. 73 *a* and 73 *b*). Accordingly, the first and simpler speck stock was discarded and the olive stock was retained. There is some uncertainty with regard to this "olive" stock, but it seems



1. Speck olive



2. Black

3. "With"

5. Streak

7. Wild-type

10. Cream II. ♂

4. Speck "without"

6. Trefoil

8. Purple

11. Cream b. ♂

9. Eosin ♂

12. Pinkish. ♂

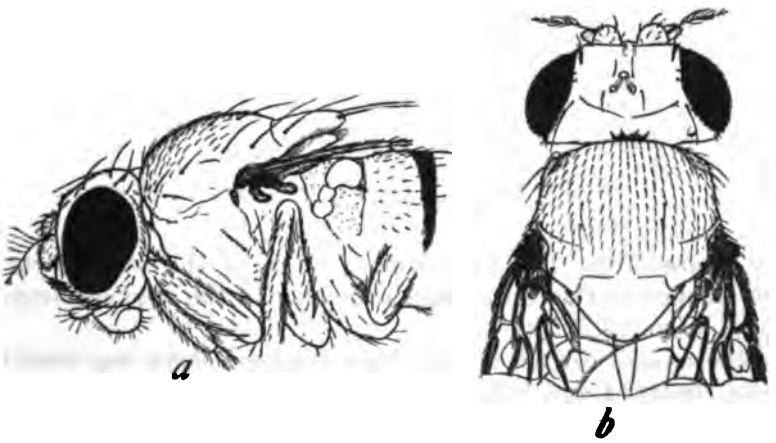
E. M. Wallace Pinx

SECOND CHROMOSOME MUTANTS OF DROSOPHILA

probable that it was derived from a stock different from the line of trident-pattern selection which gave rise to the "with" and to the "without" stocks. The early selection experiments appear to have resulted in four stocks: (1) "without" trident pattern, (2) "without" pure for the original speck, (3) "with" trident pattern, and (4) "olive" stock. The stocks of "with" and of "olive" differed in the character of the trident pattern in that the "with" is a sharp darkening of the trident pattern, while "olive" is a more diffused darkening. Subsequent analysis of the genetic behavior of these stocks showed that the "with" was a simple third-chromosome semi-dominant, while the "olive" was a trimutant stock homozygous for olive II, olive III, which is a third-chromosome recessive mutant not distinguished in appearance from olive II, and "speck," which is a second-chromosome recessive. An old figure (plate 5, fig. 4) bears out our recollection that the original speck was due to a tiny brush of hairs on the side of the thorax above the wing juncture. The new speck is a black pigment spot in the axil of the wing. The original speck seems not to have been dependable in its behavior, while the new speck has behaved with perfect regularity in inheritance. Thus, both the nature of the characters themselves and the nature of the trident mutants associated with the two "specks" lead to the conclusion that they were not the same mutants, as had been too hastily assumed. The new speck from olive stock will be referred to hereafter as speck, while the original speck will be called the old speck.

DESCRIPTION OF SPECK.

The character speck, as seen with the magnification with which we ordinarily work (about 15 diameters), is due to the presence of a minute but intensely black round speck in the axil of each wing. The speck is clearly seen from above (fig. 73 *a*) or from a little below the side (figs. 73 *b*, 75). Under the microscope the speck is seen to consist of a



TEXT-FIGURE 73.—Speck: *a*, side view; *b*, from above.

heavy deposit of pigment in the lips of a spiracle which is situated a little behind and below the juncture of the base of the wing with the thorax (fig. 73 *b*). There is also present upon the side of the scutellum and of the thorax both above and below the wing an added faint tinge of pigment or areola which seems to persist after olive has been eliminated from speck. However, speck is rarely seen without a decided olive color, because the gene for olive is very closely linked to that for speck. Since there is always the chance of crossing-over between speck and olive, and since certain other genes give roughly similar pigmentation, it is well to disregard this pigmentation and classify by means of the speck alone, which is in itself a sufficient index of the speck gene.

INHERITANCE AND CHROMOSOME OF SPECK.

During the fall and winter of 1912 considerable breeding-work with speck was carried out. The results of these experiments, particularly of the F_2 from the crosses of speck olive by black, contained the answer to the questions as to which chromosome carried the genes for speck and for olive, but the records were not carefully examined until May 1913. Meanwhile, Sturtevant had begun to work with speck (December 1912) and had shown that it was a complete and invariable recessive and behaved with regularity in inheritance.

TABLE 2.—*P_1, speck olive ♀ × wild ♂; F_1 wild-type ♀ + F_1 wild-type ♂.*

1912. Dec. —.	Wild-type.		Speck.		Olive.		Speck olive.	
	♀	♂	♀	♂	♀	♂	♀	♂
1*a*.....	41	49	....	1	37	20	25	25
1*b*.....	63	73	2	1	20	7	32	27
2*a*.....	44	23	....	2	32	39	21	23
2*b*.....	62	38	5	1	38	25	35	17
3*a*.....	38	46	1	4	11	17	32	22
3*b*.....	92	117	5	4	18	11	49	33
4*a*.....	58	75	1	2	7	2	31	16
4*b*.....	13	11	8	....	10	11	8	4
5*a*.....	68	74	5	2	18	15	28	27
5*b*.....	5	5	1	3	8	6	6	5
Total..	484	511	28	20	199	153	267	199

By means of the 2:1:1:0 ratio in the F_2 of the cross of curved to speck he showed that speck was a member of the second-chromosome group. (Jan. 13, 1913).

In the crosses carried out by Miss Wallace, speck was found to be readily classified and fully viable. Speck olive females were crossed to wild males and 10 F_2 pair cultures raised (table 2). The reciprocal cross (speck ♂ × wild ♀) was also made to the extent of 5 F_2 cultures

(table 3). These two crosses gave identical results as to the distribution of both characters and sex, which proved that no sex-linkage was involved. They may therefore be combined and considered together.

The total number of flies produced was 2,857, of which 772 or 27 per cent were speck, which is in agreement with the fact that speck is a simple autosomal recessive.

TABLE 3.—*P_1 speck olive ♂ × wild ♀; F_1 wild-type ♀ + F_1 wild-type ♂.*

1912. Dec. —.	Wild-type.		Speck.		Olive.		Speck olive.	
	♀	♂	♀	♂	♀	♂	♀	♂
a 1.....	46	67	4	....	56	29	24	30
b 1.....	44	37	....	1	35	27	29	19
b 2.....	55	45	1	1	4	.....	24	17
d 2.....	31	25	5	3	11	6	8	11
d 1.....	107	104	1	2	5	4	42	36
Total..	283	278	11	7	111	66	127	113
Totals of tables 2 and 3...	1,556		66		529		706	

The inheritance of "olive" was also followed, but since this will be treated in the following section it need only be stated here that the ratio of wild-type to olive was 9:7, which indicates two recessive body-color genes giving similar somatic effects and assorting independently of one another. One of these is olive III, (III-chromosome recessive) and the other, olive proper (II chromosome, closely linked to speck).

Speck olive males were crossed to black females, and 5 F_2 pair cultures (table 4) and 5 more from the reciprocal (table 5) were raised. The significant fact observed in these crosses was that none of the blacks were speck, which means that speck has its locus in the second chromosome. The combined data (disregarding olive) give 1,098 wild-type, 490 black, 466 speck, 0 black speck, which is an approximation to the 2:1:1:0 ratio expected from such a cross.

TABLE 4.—*P_1, speck olive ♂ × black ♀; F_1 wild-type ♀ + F_1 wild-type ♂.*

1912. Dec. —.	Wild-type.		Speck.		Olive.		Speck olive.		Black.		Black speck.	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
a 1.....	34	36	3	....	42	14	29	25	34	38	0	0
a 2.....	10	17	2	1	11	12	8	12	12	9	0	0
b 1.....	8	10	....	....	14	11	7	10	17	10	0	0
b 2.....	5	5	....	....	47	32	34	22	18	25	0	0
c 1.....	45	43	1	....	20	10	30	14	26	8	0	0
Total...	102	111	6	1	134	79	108	83	107	90	0	0

TABLE 5.—*P_1, speck olive ♀ × black ♂; F_1 wild-type ♀ + F_1 wild-type ♂.*

1912. Dec. —.	Wild-type.		Speck.		Olive.		Speck olive.		Black.		Black speck.	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
i *b*.....	37	35	....	1	31	31	35	27	21	25	0	0
ii *a*.....	27	26	1	1	63	40	32	26	44	26	0	0
ii *b*.....	54	43	....	3	40	13	16	29	31	33	0	0
iv *b*.....	57	57	....	1	17	9	29	24	38	39	0	0
v *b*.....	28	42	....	5	13	9	18	20	14	22	0	0
Total...	203	203	1	11	164	102	130	126	148	145	0	0
Totals of tables 4 and 5.....	619		19		479		447		590		0	

There was confusion in the classification of the olive in these crosses of olive to black, since flies heterozygous for black are intermediate and were often classified as olive.

LOCUS OF SPECK.

At this time it was known that the genes black, purple, vestigial, and curved were in the second chromosome alined in the order named (Morgan and Lynch, 1912; Morgan, 1912; Bridges and Sturtevant, 1914). A position for speck still further to the right was indicated by the cross-over values of table 6. Since this earlier work was

TABLE 6.—*Data upon the crossing over of speck with other second-chromosome genes, summarized from Sturtevant, 1915.*

Loci.	Total.	Cross-overs.	Per cent of cross-overs.
Black speck.....	223	110	49.3
Vestigial speck...	1,446	520	36.0
Curved speck....	1,007	262	26.0

TABLE 7.—*P_1, purple curved speck ♂ × wild ♀; B. C., F_1 wild-type ♀ × purple curved speck ♂ from stock.*

1914, Aug. 24.	$\frac{pr\ c\ sp}{}$		$\frac{pr}{c\ sp}$		$\frac{pr\ c}{\ \ sp}$		$\frac{pr\ \ sp}{c}$	
	Purple curved speck.	Wild-type.	Purple.	Curved speck.	Purple curved.	Speck.	Purple speck.	Curved.
452.....	99	97	25	28	54	47	4	1
481.....	69	74	15	24	26	27	.....	2
507.....	24	53	14	20	23	22	4	3
508.....	51	56	17	20	26	22	1	4
Total.....	243	280	71	92	129	118	9	10

TABLE 8.—*A summary of the cross-over data involving speck.*

Loci.	Total.	Cross-overs.	Per cent.	Date.	By —	Reference.
				1915		
Star speck....	369	185	50.1	June 28	Bridges.....	S'; $\frac{S'}{s_g}$ B. C.; 1806–'08.
	6,766	3,264	48.3	July 11	Do.	S'; $\frac{S'}{p_r\ c\ s_g}$ B. C.; 1sts; 1836–'94.
	7,135	3,449	48.4			
				1914		
Streak speck..	462	242	52.3	May —	Muller.....	Am. Nat. '16, p. 422.
Dachs speck...	462	231	50.0	May —	Do.	Do.
				1913		
Black speck...	223	110	49.3	Oct. 16	Sturtevant...	Zeit. f. i. A. u. Ver. '15, p. 245.
				1914		
	462	216	46.8	May —	Muller.....	Am. Nat. '16, p. 422.
	685	326	47.6			
Purple speck..	462	218	47.2	May —	Muller.....	Do.
	952	410	43.1	Aug. 24	Bridges.....	s_p; $p_r\ c\ s_p$ B.C.; 452–508.
	2,625	1,116	44.4	Oct. 24	Do.	a; $p_r\ a\ s_p$ balanced B.C.; 637–686.
				1915		
	6,766	3,130	46.3	July 11	Do.	S'; $\frac{S}{p_r\ c\ s_g}$ B.C.; 1sts; 1836–'94.
				1916		
	259	95	36.7	Feb. 7	Do.	l_{IIa}; $\frac{p_r\ s_g}{l_{IIa}}$ F_2; 3168.....
	565	279	49.4	Feb. 8	Do.	l_{IIa}; $p_r\ c\ s_g$ F_2; 3203–8.
	356	176	49.4	Feb. 29	Do.	l_{IIa}; $p_r\ p_z\ s_g$ F_2; 3535.....
	11,985	5,474	45.7			
				1913		
Vestigial speck	1,446	520	36.0	Mar. 14	Sturtevant...	Zeit. f. i. A. u. Ver. '15, p. 245.
	146	40	27.4	Oct. —	Do.	Zeit. f. i. A. u. Ver. '15, p. 287.
				1914		
	462	178	38.5	May —	Muller... ...	Am. Nat., '16, p. 422.
	2,054	738	35.9			
				1913		
Curved speck..	1,007	262	26.0	Mar. 10	Sturtevant...	Zeit. f. i. A. u. Ver. '15, p. 245.
	223	71	31.8	Oct. 16	Do.	Zeit. f. i. A. u. Ver. '15, p. 247.
				1914		
	462	150	32.5	May —	Muller.....	Am. Nat., '16, p. 422.
	952	266	27.9	Aug. 24	Bridges.....	s_p; $p_r\ c\ s_p$ B.C.; 452–508.
				1915		
	6,766	2,062	30.5	July 11	Do.	S'; $\frac{S'}{p_r\ c\ s_g}$ B.C. firsts; 1836–'94.
				1916		
	632	226	35.7	Feb. 8	Do.	l_{IIa}; $p_r\ c\ s_g$ F_2; 3203–8.
	10,042	3,037	30.5			
Plexus speck ..	327	29	8.9	Feb. 29	Do.	l_{IIa}; $p_r\ p_z\ s_g$ F_2; 3535.....
				1914		
Arc speck.....	2,625	156	5.9	Oct. 24	Do.	a; $p_r\ a\ s_g$ balanced B.C.; 637–686.
Blistered speck	36	3	8.3	Feb. 27	Do.	b_g; $\frac{b_g}{s_g}$ B.C.; 72....
Speck balloon .	462	2	0.4	May —	Muller.....	Am. Nat., '16, p. 422.

finished, very large amounts of additional accurate data have been collected upon the cross-over relations of speck with other second-chromosome genes. These data appear in the tables of the sections following this (see especially star).

Only one of these later experiments had as the main object the more exact determination of the locus of speck. This experiment (table 7) was a triple back-cross for the three loci—purple, curved, and speck—and gave a curved speck cross-over value of 27.9 per cent on the basis of the 952 flies, of which 266 were cross-overs between curved and speck. The base of reference in the determination and mapping of the locus of speck is curved, which is the nearest locus accurately mapped in relation to black, the primary base of the entire second chromosome.

For the sake of convenience a summary of all the cross-over data in which speck is one of the loci involved is given in table 8. In calculating the locus of any mutant one must consider not only this direct-linkage data, but also the whole mass of data on the other loci of the same chromosome, and especially the information upon the amount of double crossing-over and coincidence in the various regions. By this method speck has been mapped at a locus 31.6 units to the right of curved, which is its immediate base of reference, or, referring back to star as the zero-point, speck is at 105.1.

VALUATION OF SPECK.

Speck is at present one of the most generally useful and used of the second-chromosome mutants, first, because of the perfect accuracy, ease, and speed with which the recessive character is separable from wild-type; secondly, because it can be used in experiments with any of the other second-chromosome characters (including black) without masking effects or confusion in the classification; thirdly, on account of the value of the position of its gene near the right-hand end of the second chromosome, speck being by far the most workable mutant in that general region; and finally, because its viability, its productivity, and its fertility are above reproach, and it is singularly free from such bad habits as getting drowned, or stuck in the food, or refusing to be emptied from the culture bottle, etc., which alienate the affections of the experimenter from certain other mutants. Speck is to be commended for students' use, but care should be taken that the character is clearly recognized.

LITERATURE OF SPECK.

The more important papers referring to speck are: Morgan, 1910, describing its origin and giving the irregular breeding results already commented upon above; Sturtevant, 1915, giving the data of table 6, by means of which the locus of speck was first worked out; and Muller, 1916, speck being one of the mutants used in the progeny test of the linkage of second-chromosome genes.

OLIVE.

ORIGIN AND STOCK OF OLIVE.

The origin and early history of the stock called "olive," because of the body-color present, is only partly a matter of record, though the account given in the section on speck is substantially correct. To repeat: In the fall of 1909, Morgan started selection on stocks of wild flies that were throwing individuals with extra-dark trident patterns. One line of selection eliminated this variation, and the resulting "without" stock represented the original wild stock before the occurrence of the "with" mutation. It was in this "without" stock that the old speck was found. The selection in the opposite direction isolated the third-chromosome semi-dominant mutation "with" (plate 5, fig. 3). The "olive" stock appears to have resulted from selection carried out on a stock different from that which gave rise to "with." The trident pattern of the "olive" stock is dark, but is not distinct, being submerged in a general olive color that suffuses the thorax. The "olive" stock was obtained about May 1910, and had been kept in the stock room about a year when it was noticed that it was pure for speck.

CHROMOSOME AND LOCUS OF OLIVE.

During the fall and winter of 1912 several crosses were carried out by Miss Wallace with the "olive" stock. "Olive" crossed to wild gave wild-type offspring which were inbred in pairs to give F_2. This cross and the reciprocal were both made and gave the same kind of F_1 and F_2 results in the distribution of both characters and sex, showing that no important sex-linked modifiers of body-color were present. The combined F_2 counts (2,857 flies) gave a very perfect 9 : 7 ratio of gray to "olive" (1,622 : 1,235), showing that the "olive" stock contained two recessive dark body-colors whose genes assorted independently (tables 2 and 3). A further separation of the "olive" classes into the component two single and the double recessive forms was not attempted. That one of the recessives (olive II) was carried in the second chromosome was proved by the strong linkage olive showed with speck (tables 2 and 3). There were only 2.3 per cent of flies that were speck not-olive. The whole observed distribution corresponds to about 14.3 per cent of crossing-over between speck and olive. It is certain that this value is far too high due to the difficulty in classifying the olive. Our later experience has been that olive is probably less than a unit distance from speck, and probably to the right, which give an approximate locus of 106 when referred to star.

Very rarely have we secured speck flies that we consider free from olive. The original stock of speck was probably not-olive, and in a certain experiment made by Sturtevant (1915) it seems likely that the

great difficulty of classification was due to a speck from which olive had been lost by crossing-over. On one other occasion speck has been found which seemed to be without olive. We believe that most of the few flies classified as "speck not-olive" in the F_2 of olive by wild were either young flies in which the olive was not yet developed sufficiently to be surely classifiable or were fluctuants extreme enough to cause trouble, neither of the two olives being sufficiently dark or constant in color to be invariably separable from the wild type.

Throughout our discussion of speck and olive it has been assumed that the olive color which is nearly always seen in speck flies is due to a separate gene, and while this is probable, the evidence is by no means conclusive. It may be that the two characters are the products of a single gene, and that the supposed cases of "speck not-olive" have been due on occasion to wrong classification of the poor character olive, or to the action of minus modifying genes, or to a new speck allelomorph which differed in this regard from the old. A further careful investigation would be required to settle this question.

VALUATION OF OLIVE.

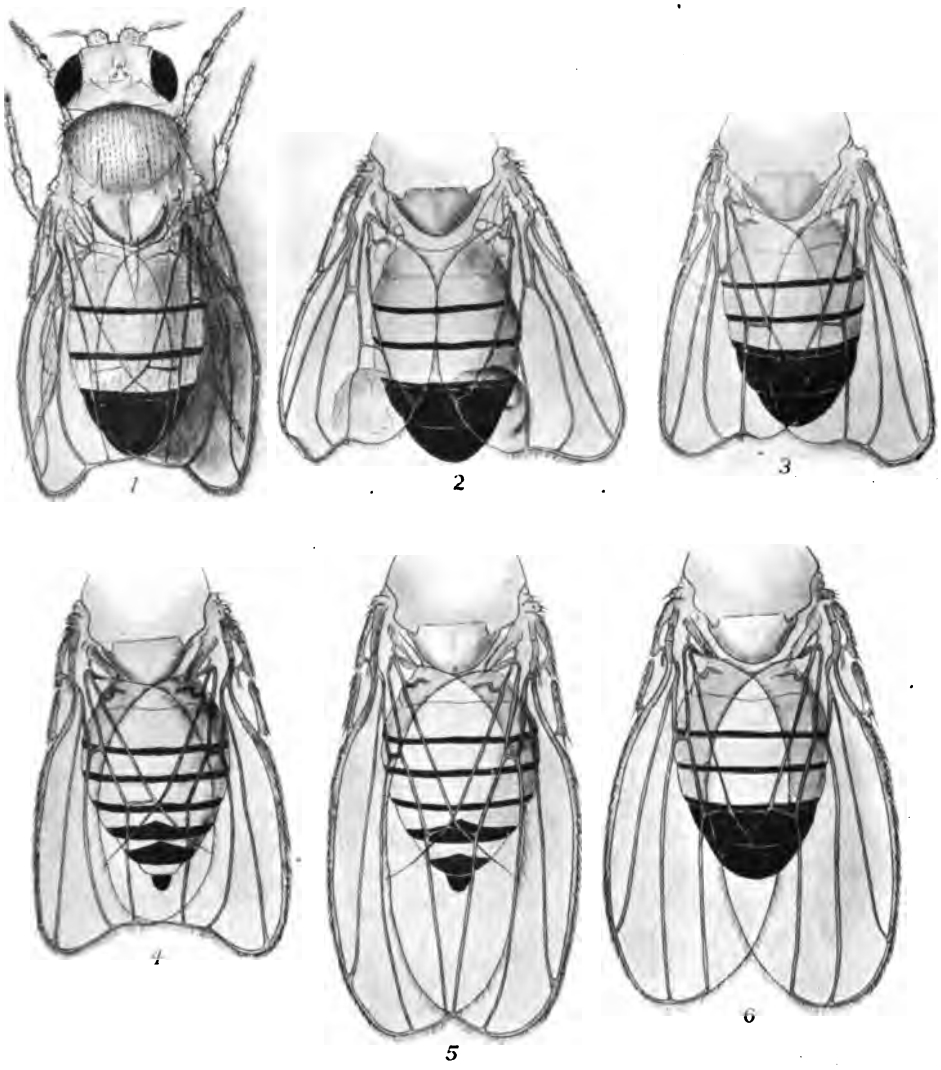
Olive II is of no value for further work, since the character is not sufficiently distinct from wild-type so that the normal fluctuations in these two body-colors do not overlap, and classification is accordingly both difficult and inaccurate. Olive is subject to a general objection to the usefulness of all faint body-colors, namely, the fact that body-colors take some time after hatching for their full development, and in cases where the final difference is not great the intermediate stages are unpleasant to work with and a source of error. A further defect in the value of olive is the uncertainty as to whether the character may not be found to be only another expression of the speck gene. Furthermore, in case the investigation should prove that two linked but separable genes were involved, the usefulness of olive would not thereby be improved, since olive could be used for no purpose that could not be better met by the use of speck or one or another of the mutations whose genes are in this same region. As far as we have been able to observe, the presence of olive has had no detrimental effect upon the viability or other qualities of speck.

TRUNCATE (*T'*).

(Plate 6, figures 1 to 6.)

ORIGIN OF TRUNCATE.

One of the early mutations (beaded, May 1910) had been run for seven generations in stock cultures when a fly appeared (August 1910) whose wings were somewhat obliquely truncated and somewhat


1
2
3
4
5
6

shorter than usual (Morgan, 1911). This fly when bred to his wild-type sisters produced about 10 per cent of offspring whose wings showed a slight or moderate amount of truncation (plate 6, figs. 4 and 5). Some of these truncated flies bred together produced in F_2 nearly 50 per cent of truncated. For several months it was impossible by selection to raise the percentage of truncates much above 50 per cent, though there was an increase in the shortness of the wings of the truncates that did occur. But later certain cultures gave much higher percentages, and selection started at this point and continued for about a year established a stock in which the percentage of truncates was not far from 90 per cent. Above this level it was impossible to maintain gains.

Along with this increase in the percentage of truncate individuals, several other changes were observed to be going on. There was an increase in the number of flies which were sterile and gave no offspring; at times about 50 per cent of the pairs were sterile. There was a marked decrease in the productivity of those pairs which did give offspring. These two facts made the stock very difficult to carry on, except in large mass cultures. In those cultures in which the percentage of truncates was high, the amount of the truncation was great; *i. e.*, many of the flies had extra-short wings, some wings being even shorter than the abdomen (plate 6, figs. 1 and 2). It was found that if these short-winged flies were used in carrying on the stock, the percentage of truncates was higher. The flies whose wings were slightly truncated gave under 90 per cent of truncated, while those flies whose wings showed no truncation either gave no truncates or less than or about 50 per cent of truncates. In all of these cultures more of the females than of the males showed the truncate character; that is, truncate is "partially sex-limited" in its expression.

At this point Mr. E. Altenburg took up the selection of truncate and confirmed the points just stated. Later, Altenburg and Muller (Mechanism of Mendelian Inheritances; also mss.) sought to analyze the truncate stock by the method of linkage to find the cause of the continued appearance of normal flies and of the variations in the extent of the truncation. Their tests showed that truncate is primarily due to a dominant gene in the second chromosome. This gene is lethal when homozygous, so that pure-breeding stock can not be obtained. When heterozygous, this gene is capable of producing only a moderate truncation and fluctuates in expression, so that most of the flies having the truncate gene fail to show the character at all. But in the selected stock there are certainly two (one in the first and one in the third) and probably more genes whose effect is to increase the amount of truncation, whereby both a greater proportion can be detected and the wings of those which do show truncation are shortened.

Such intensifiers could theoretically only raise the amount of truncation to the extent that every fly carrying the truncate gene can be

distinguished from the wild type, and because of the lethal action of the truncate gene this can not exceed two-thirds of the flies.

If some of these modifiers could themselves produce a moderate truncation, then the percentage would pass beyond 67, but there is evidence that the modifiers are unable to produce truncation in the absence of the chief gene.

TRUNCATE LETHAL.

Since at least one-third of the flies must look like wild flies rather than truncates, their non-appearance in the highly selected stock is to be accounted for by the assumption of a lethal gene which is carried in the II chromosome, homologous to that carrying the truncate gene. This condition has not been proved by testing, but the action of autosomal lethals is well understood and a similar case of an autosomal lethal giving an apparently pure-breeding stock when balanced against an autosomal dominant which is itself lethal when homozygous (beaded) has been demonstrated by Muller.

It is now clear what is the meaning of the early history of the truncate stock which was for so long a puzzle. The original truncate fly was heterozygous for the dominant truncate gene. In a cross to wild-type sisters only 10 per cent of the offspring showed the truncate character, though half of them carried the truncate gene. This low power of expression of the character was due to the lack of effective modifiers, most of which were recessive.

The first period of inbreeding and selection resulted in the collecting of whatever modifiers were present, and by the approach to homozygosis allowed more of the recessive ones to show their effect. But by such means the proportion of truncate could not be advanced beyond 67 per cent and was not advanced much beyond 50 per cent.

The limit of 67 per cent imposed by the lethal nature of the primary truncate gene must have been broken down by the occurrence (February 1911) of the lethal mutation in the chromosome homologous to that carrying the primary truncate gene. The second period of selection then increased the percentage of truncates by increasing the prevalence of this lethal in the stock. The limit which this second selection approached was the condition in which every second chromosome which was not carrying the truncate gene should carry the lethal. By this means all the non-truncate flies would be eliminated except those saved by crossing-over. If the lethal were 20 units away from truncate, measured along the second chromosome, then 10 per cent of not-truncate flies should survive. The very low productivity of the truncate stock observed at this time was the result of the action of the two lethals—all homozygous truncates as well as all homozygous lethals dying as early zygotes; also the modifiers tending to produce infertility when homozygous. While the proportion of truncates was

limited at the two values, 67 and 90 per cent, the accumulation of the modifiers continued to make the wings shorter, and the shortest individuals gave the highest percentage of truncates, since they were on the average the ones carrying most modifiers.

The increase in the number of completely sterile individuals was independent of the other changes and due to a mutation which occurred early in the selection. Hyde (1914) was able to eliminate this sterility from the truncate stock by breeding for some generations from those families which showed least sterility. But there was no rise in productivity parallel to the elimination of the sterility. This sterility behaved as a recessive in the F_1 out-crosses, and in F_2 reappeared, but in such proportion and distribution as to suggest that it also was complex.

The fact that the main gene for truncate is in the second chromosome was established by Muller and Altenburg through the non-occurrence of cross-overs in back-cross tests of males heterozygous for truncate and black. In back-cross tests of similar females there was somewhat under 25 per cent of crossing-over. A back-cross test showed that there is somewhat over 25 per cent of crossing-over between star and truncate, and this information, in connection with the known distance of about 50 between star and black, showed that truncate is located about midway between star and black and slightly nearer to black. Since the amount of data secured in these tests is not large and because truncate is so elusive a character, the location is not precise, though the locus is probably not far from 28 (see Snub).

TRUNCATE REOCCURRENCES.

Many of our mutant characters have made their appearance more than once, and occasionally under circumstances which make it certain that there has been a new occurrence of the same mutative process that was responsible for the original appearance of the character. In other cases it is probable that the character is not reappearing because of a fresh mutation, but that the original mutant gene had been introduced in some previous cross and has been unsuspectedly present for several generations but has been unable to appear because of the way in which the crossing has been carried on. In still other cases a character appears which resembles very closely another already known character, but the two are the result of mutations in entirely different loci which are often in different chromosomes. Thus, we have at least half a dozen mutant characters of the "genus" pink which are so similar as to be practically indistinguishable in mixtures, yet which are dependent on entirely distinct genes. Characters of the genus truncate are the most frequently recurring of any, with the possible exception of the beadeds. These two kinds of character have both come up in the breeding work three or four times each year.

In most of these cases the character is also "specifically" truncate, and usually due to the original truncate gene rather than to a fresh mutation. In the absence of its usual intensifiers truncate may lurk unsuspected in a stock or an experiment for many generations and is difficult to eliminate. For this reason it is practically impossible to be certain in any unexpected case of truncate appearance that there has been a fresh mutation. It is not ordinarily profitable to pay any attention to these appearances of truncate, but in two instances in which, because of the pedigree, there was less than the usual likelihood that the truncate was due to the original gene, tests were made, and in both cases the character was found to be either truncate or else a very similar allelomorph, though these tests could throw no new light on the question of whether the gene were the original or a fresh truncate mutation.

SNUB.

The first of these tests was made by Muller (unpublished). The second case appeared in the ninth generation of some experiments on "duplication." A cross had been made between a female with the new sex-linked recessive wing-character "cut" and a not-cut male (February 17, 1916, culture 3338, Bridges). All the daughters were expected to be wild-type and all the sons cut; but 9 of the 77 daughters were seen to be slightly truncated, the character being called "snub," while some 18 of the 67 sons were cut with shortened and blunted wing ends (cut snub).

One of these cut-snub males outcrossed to a wild female gave about a quarter of the F_1 flies with the snub or truncate character. This result showed that the character was a dominant, though a "poor" one; that is, not all the flies genetically alike (heterozygotes) showed the character somatically. The snub appeared among the F_1 males as well as among the females, and this fact showed that the character was non-sex-linked, for had it been sex-linked it could have appeared only among the daughters of the above cross.

When snub flies were bred together, the result was usually an approximation to a 2 snub: 1 not-snub ratio, often with the snubs below expectation because of the above-noted occasional failure of heterozygous snubs to show the character somatically. This ratio and the fact that it proved impossible to obtain a pure-breeding snub stock suggested that the mutant was lethal when homozygous, as are most of our dominants. In cut-snub stock the approximation to the 2 : 1 ratios was much closer, and it seems certain that the character cut favors the differentiation of snub (see cultures 1 to 10, table 9). We are well acquainted with such intensifiers or modifiers in other cases, and truncate itself was known to be very susceptible to intensification. A few of the cut-snub pairs gave nearly all of the flies snub (see especially 10 and 12, table 9, Morgan), and it seems probable that in these

cases a lethal was present in the homologous chromosome. Autosomal lethals are very plentiful and have been clearly demonstrated in many other cases, though in this case no further test has yet been made of the correctness of this explanation.

That the same kind of modifiers were present in the snub stock as were responsible for the short-winged types of truncate appeared certain from the rather sharp differences between the cut snubs which occurred in the inbred stock. While most of the cut snubs were of the type described, in which the wing is nearly as long as the normal cut but differently shaped at the tip, certain ones were much shorter and the oblique truncation was very marked. These shorter ones, just as in the case of the selected truncate stock, were most numerous in those cultures in which the expected 2 : 1 ratio was most closely approached. Pair 10 of table 9 showed a ratio of 31 short snubs to 63 of medium or slight truncation to 46 which showed no truncation; this rather close approach to a 1 : 2 : 1 ratio suggested that probably only one such modifier was present in the cross.

TABLE 9.—*The offspring given by pairs of cut snub flies from the stock of cut snub.*

Culture No.	Cut snub.	Cut.
1	30	18
2	33	16
3	33	15
4	24	13
5	30	15
6	13	10
7	45	13
8	24	15
9	51	25
10	94	46
	377	186
11	27	2
12	25	2
	52	4

All of the characteristics of snub thus far found have agreed exactly with those of "specific" truncate. If it should be found that the chromosome locus of snub were the same as for the original truncate, then we should conclude that the mutation is specifically truncate.

Because of the fluctuating nature of the dominance of snub and its easy modification, a direct linkage experiment offered difficulties. A more exact method would be to establish the lethal nature of the truncate-snub compound. This could be done by showing that the F_1 ratio obtained by crossing truncate by snub was a derivative of a 2 : 1 instead of a 3 : 1 ratio. The observed ratio of 174 truncate to 132 not-truncate in the F_1 from this cross would somewhat favor the view that the ratio is 2 : 1 rather than 3 : 1, and that snub is therefore truncate (table 10, Morgan). But here again the uncertainty that the number actually showing the truncate character would be a close enough approach to the number heterozygous for truncate, so that we could decide whether we were really dealing with a 2 : 1 or a 3 : 1 ratio made the results of such experiments of doubtful value.

It was recalled that cut had acted as an intensifier of snub, so that a larger proportion of cut flies showed the snub character than was the case among the not-cut flies. Advantage of this fact was taken by

crossing a cut-snub female to truncate males, in which case all the sons were cut. Among these cut sons the ratio of snub to normal was almost exactly 2 : 1 (table 11). The fact that this experiment gave a close approach to the ratio expected if the truncate-snub compound is lethal could not be accepted as proving that theory, because there might still be enough flies failing to show the truncation, so that the ratio is really the normal 3 : 1 ratio.

TABLE 10.—*F_1 ratios obtained from crosses of truncate ♀ × snub ♂ (cultures 1 to 4), and snub ♀ × truncate ♂ (cultures 5 and 6).*

Culture. No.	Truncate-like.	Not truncate-like.
1	11	8
2	51	45
3	50	48
4	5	12
5	43	16
6	14	3
Total	174	132

TABLE 11.—*F_1 ratios obtained from crosses of cut snub ♀ × truncate ♂ (pairs).*

1917 Jan.	Wild-type ♀.	Truncate-like ♀.	Cut ♂.	Truncated-cut ♂.
A	48	57	38	56
B	39	66	25	62
C	34	71	29	59
D	22	59	31	66
E	28	73	23	56
F	39	39	16	35
Total	210	365	162	333

The scheme finally followed eliminated all classification of both truncates and snubs and depended for identification upon the readily classifiable character star and upon the assumed lethal nature of the truncate-snub compound. By mating star to truncate, flies can be obtained carrying the star gene in one II-chromosome and truncate in the other $\left(\frac{S' \quad +}{+ \quad T'}\right)$. Two such flies mated together would give a 2 star to 1 not-star ratio, unless homozygous truncate were lethal. But since homozygous truncate is known to be lethal, this ratio becomes modified by the death of most of the not-star flies. A few not-star offspring will survive because of crossing-over in the female $\left(\frac{S' \quad +}{+ \quad T'} \rightarrow \frac{S' \quad T'}{+ \quad +}\right)$. There is a precise relation between the amount of this crossing-over and the number of not-star flies which

appear. If x is the percentage of cross-over gametes, and $100x$ of the non-cross-over gametes, then the ratio of star to not-star is $200x : x$.

Obviously, if snub is truncate, then by mating such a star-truncate heterozygote by a similar star-snub heterozygote, one should get this same $200x : x$ ratio of star to not-star. That is, the occurrence of a ratio of star to not-stars in which the not-stars are less numerous than in the ordinary 2 : 1 ratio would mean that snub is truncate, and by calculation from the observed ratio we can find out how far the locus of truncate is from star. The experiment as carried out gave (table 12, Wallace) four cultures in which the ratio was significantly different from 2 : 1. The total of stars was 950 and of not-stars 149, or a ratio of 6.3 : 1. From this we may conclude that snub is truncate or an allelomorph so similar in its obvious characteristics that to demonstrate a difference would require a refined biometrical study or elaborate 'interaction' tests.

TABLE 12.

Culture No.	Stars.	Not-stars.
1	199	27
2	264	21
3	300	84
4	187	17
Total	950	149

In table 12 will be found the ratio of star to not-star given by crosses of females heterozygous for star and truncateto males heterozygous for star and snub.

The solution of the proportion $200x : x : 950 : 149$ gives a value of 27.1 for x; that is, there is 27.1 per cent of crossing-over between star and truncate. Nearly 1 per cent should be allowed for double crossing-over within the distance from star to truncate, or the map distance should be about 28.0 on the basis of this data. This position also agrees with what is known of the location of truncate.

INTENSIFICATION OF TRUNCATE BY CUT.

That cut intensifies the original truncate in the same manner as it does snub is shown by the results of a test of this point (table 13). When a cut female was crossed to a truncate male the ratio of not-truncate to truncate among the males was 1:0.79; that is, quite a

TABLE 13.—*Intensification of truncate by cut—P_1, cut ♀ × truncate ♂.*

1917, Jan.	Wild-type ♀.	Truncate ♀.	Cut ♂.	Truncated cut ♂.
I	81	44	61	42
II	66	15	44	41
Total..	147	59	105	83

close approach to the expected 1 : 1; but among the sisters, which were not cut, this ratio was 1 : 0.40, which means that about 43 per cent of the females genetically truncate failed to show the character. The intensification by cut is more extensive than appears at first

glance, because normally the males show a considerably smaller percentage of truncate than do the females. The difference in culture II was especially striking.

A recent census of the truncate stock, which has been run for about 5 years under selection not especially rigorous, showed about 17 per cent of wild-type flies (table 14). The truncate flies were of various degrees of shortness, of which the most frequent was that known as "intermediate" (corresponding to fig. 4 of Plate 6). The very short truncates were not especially numerous, although in selecting the parents each generation they had been preferred. Table 14 gives the census of truncate stock (May 1917).

TABLE 14.

	♀	♂
Wild-type	37	104
"Slight"	96	46
"Long"	62	50
"Intermediate"	164	146
"Short"	52	63

BLACK (*b*).

(Plate 5, figure 2.)

ORIGIN OF BLACK.

The first workable body-color mutation, black, was found by Morgan, October 1910, in the F_2 of a cross between miniature and wild flies (Morgan, 1911).

DESCRIPTION OF BLACK.

When black flies are freshly hatched little black color has developed on the body, though the legs and feet are darker than normal. Within a few minutes after hatching the color has deepened so that the head, thorax, and abdomen are a clean, fresh, greenish black, more intense on the thorax than on other parts. This color becomes progressively darker with age. The wings, after expanding, also become much darker, and along each side of the veins a broad band of pigment begins to develop and becomes conspicuous in old flies.

SEMI-DOMINANCE OF BLACK.

While the fly heterozygous for black is noticeably darker than the wild-type, this separation can not be made completely, although it is occasionally made use of for special purposes (see sections on Jaunty, p. 162, and Apterous, p. 237). Black is generally, therefore, treated as a recessive, and the separation of black from the heterozygote is easy and entirely accurate.

CHROMOSOME CARRYING BLACK.

Black is a member of the second-chromosome group by definition.

As soon as it had been established that the loci for the sex-linked mutations were capable of being mapped in definite positions (Sturte-

vant, 1912), this same procedure was applied to the non-sex-linked mutations. The cytological work of Miss Stevens had shown that there were at least three autosomes in *Drosophila*, and it was expected that a group of linked genes would be found to correspond to each of these. At this time there were only two non-sex-linked mutations—black and pink—whose inheritance had been worked out by Morgan and whose behavior was definitely known to be Mendelian and normal. The next point was to establish the relation of these mutants to each other in inheritance. This was done by raising an F_2 from the cross of black by pink. The F_2 ratio was quite clearly that of independent inheritance, since it approximated 9 : 3 : 3 : 1, with the double recessive present in as large numbers as expected (Sturtevant, February 1, 1912). Sturtevant soon showed by means of back-cross tests that there was no appreciable linkage between black and pink. Provisionally, black and pink were assumed to be in separate chromosomes—the second and the third. The second chromosome is arbitrarily that chromosome which carries the gene for black, and any other genes that may be found to be linked to black; similarly, the third chromosome is defined as that chromosome which carries the gene for pink, and all other genes found to be members of the linkage group containing pink.

Soon after the black pink F_2 had given the first autosomal independence, an F_2 between black and curved demonstrated the first autosomal linkage. As soon as this linkage was observed (March 4, 1912) definite plans were made to test the linkage relations (chromosome and locus) of all the autosomal mutants thus far found, making full use of the back-cross method. (See Bridges and Sturtevant, 1914, p. 205). By the middle of July it was known as a result of these tests that besides black and curved, purple, vestigial, balloon, blistered, jaunty, and arc were in this second chromosome.

LOCUS OF BLACK.

The locus of black was taken as the base of reference in the mapping of these other genes. Since curved was the first mutant known to be in the second chromosome with black, its locus determined the direction along the chromosome which was to be defined as "to the right" (black curved). The loci of all the other mutants just mentioned were later found to lie on the same side of black as curved does, so that black was the locus farthest to the left, and the natural zero-point of the map. Black is now the real base of reference in the mapping of the entire second chromosome, and all other genes are plotted in relation to it, either directly in the case of those genes nearby (dachs, jaunty, purple, vestigial, etc.), or indirectly by being located with reference to certain important genes (star, curved, speck, etc.), whose positions with regard to black have been so well established that they in turn can safely be used as secondary bases.

TABLE 15.—*Summary of the cross-over data involving black.*

Loci.	Total.	Cross-overs.	Per-cent.	Date.	By —	Reference.
Star black....	1,352	522	38.6	Jan. 11, 1915	Bridges....	p_z; $\frac{S'}{b\ p_z}$ B.C.; 1921–'24.
	496	203	40.9	Oct. 23, 1915	Do.	f_r; $\frac{S'}{b\ f_r}$ B.C.; 2282–'84.
	865	315	36.4	Oct. 26, 1915	Do.	v_g^n; $\frac{S'}{b\ v_g^n}$ B.C.; table 123, this paper.
	690	266	38.6	Dec. 22, 1915	Do.	d_1; $\frac{S'\ d_1}{b}$ B.C.; 2679–7085.
	13,104	4,944	37.7	Dec. 5, 1916	Plough...	J. E. Z., '17, p. 147; temperature; $\frac{S'}{b\ c}$ B.C.; tables 7 (22°), 8_3 (27°), 8_6 (22°), 11_1 (22°), 17_2.
	16,507	6,250	37.9			
Streak black..	462	120	26.0	May —, 1914	Muller.....	Am. Nat., '16, p. 422.
Dachs black ..	338	82	24.3	Mar. 18, 1913	Bridges....	d; $d\ b\ F_2$; II 34–36.
	933	163	17.5	June 30, 1913	Do.	d; $d\ b$ B.C.; II 40–II 98r.
	4,892	874	17.9	Dec. 10, 1913	Do.	d; $d\ b\ v_g$ balanced B.C.; II 114–II 138.
	462	77	16.7	May —, 1914	Muller.....	Am. Nat. '16, p. 422.
	6,725	1,196	17.8			
Squat black...	82	9	11.0	Apr. 3, 1911	Bridges....	Sq; $\frac{Sq}{b\ p_z}$ B.C.; 4044.
Black jaunty..	462	1?	0.2?	May —, 1914	Muller.....	Am. Nat. '16, p. 422.
Black purple..	773	38	4.9	Dec. 12, 1912	Bridges....	pr; $b\ p_r$ B.C.; C 174–II 2.
	3,934	212	5.4	Aug. 28, 1913	Do.	pr; $b\ p_r\ c$ B.C.; 1sts; II 58–II 88.
	5,001	322	6.4	Jan. 9, 1914	Do.	pr; $b\ p_r\ v_g$ balanced B.C.; II141–674.
	462	26	5.6	May —, 1914	Muller.....	Am. Nat. '16, p. 422.
	36,622	2,214	6.0	Jan. 5, 1915	Plough....	J. E. Z., '17, total $b\ p_r\ c$ B.C.
	2,139	214	10.0	Mar. 5, 1917	Do.	J. E. Z., '17, total $b\ p_r\ v_g$ B.C.
	48,931	3,026	6.2			
Black-l_{IIa}.....	166	22	13.0	Jan. 15, 1916	Bridges....	l_{IIa}; $b\ l_{IIa}$ F_2; 2840, '59, '63.
Black vestigial.	3,499	632	18.1	Sept. 10, 1912	Morgan ...	Biol. Bull. '14, p. 197; $\frac{b}{v_g}$ B.C.
	1,268	217	17.1	Sept. 10, 1912	Do.	Biol. Bull. '14, p. 198; $b\ v_g$ B.C.
	694	169	24.4	June 8, 1913	Sturtevant.	Zeit. f. i. A. u. V.'15, p. 286; $b\ v_g\ c$ balanced B.C.
	4,892	806	16.5	Dec. 10, 1913	Bridges....	d; $d\ b\ v_g$ balanced B.C.; II 114–II 138.
	5,001	815	16.3	Jan. 9, 1914	Do.	p_r; $b\ p_r\ v_g$ balanced B.C.; II 141–674
	462	78	16.9	May —, 1914	Muller.....	Am. Nat. '16, p. 422.
	450	99	22.0	Nov. 17, 1915	Bridges....	v_g^n; $\frac{S'}{b\ v_g^n}$ B.C.; table 123, this paper.
	2,139	477	22.3	Mar. 5, 1917	Plough....	J. E. Z., '17, $b\ p_r\ v_g$ B.C.
	1,748	285	16.3	May —, 1917	Gostenhofer	$\frac{b}{v_g}$ B.C.; students' records.
	20,153	3,578	17.8			

TABLE 15.—*Summary of cross-over data involving black*—continued.

Loci.	Total.	Cross-overs.	Per-cent	Date.	By —	Reference.
Black curved..	7,419	1,717	23.1	Jan. 13, 1913	B. & Stutt.	Biol. Bull. '14, p. 209; *b c* and $\frac{b}{c}$ B.C.
	260	69	25.5	Jan. 13, 1913	Do.	Biol. Bull. '14, p. 208; *b c* F_2.
	253	66	26.1	Jan. 13, 1913	Do.	Biol. Bull. '14, p. 212, $\frac{b}{c}$ B.C.
	402	120	29.9	June 8, 1913	Sturtevant.	Zeit. f. I. A. u. V.; '15, p. 247; *b* v_g *c* B.C.
	3,934	839	21.3	Aug. 24, 1913	Bridges....	p_7; *b* p_7 *c* B.C.; 1sts; II58–II88.
	223	63	28.2	Oct. 16, 1913	Sturtevant.	Zeit. f. I. A. u. V., '15, p. 247, *b c* s_p B.C.
	462	106	22.9	May —, 1914	Muller.....	Am. Nat. '16, p. 422.
	36,622	8,598	23.4	Jan. 5, 1915	Plough....	J. E. Z. '17, *b* p_7 *c* B.C. totals.
	13,104	2,659	20.3	Dec. 13, 1915	Plough....	J. E. Z. '17 $\frac{S'}{b\ c}$ B.C. controls.
	62,679	14,237	22.7			
Black plexus..	1,026	417	40.6	Jan. 1, 1915	Bridges....	p_2; *b* p_2 B.C.; 1084–'99.
	1,352	576	42.6	July 20, 1915	Do.	px; $\frac{S'}{b\ p_2}$ B.C.; 1921–'24.
	82	38	46.4	Apr. 3, 1916	Do.	S_6; $\frac{S_6}{b\ p_2}$ B.C.; 4044.
	2,460	1,031	41.9			
Black fringed.	496	211	42.5	Oct. 23, 1915	Bridges....	f_7; $\frac{S'}{b\ f_7}$ B.C.; 2282–'84.
Black arc.....	798	286	35.9	Dec. 13, 1912	Bridges....	*a*; *b a* B.C.; C 172–II 3.
	6,794	2,951	43.4	Aug. 4, 1914	Do.	m_7; *b a* m_7 balanced B.C.; 364.
	7,592	3,237	42.6			
Black blistered	224	93	41.5	Nov. 4, 1913	Bridges....	b_2; $\frac{b}{B_2}$ B.C.; II 102——.
Black pinkish.	736	371	51.4	Sept. 22, 1914	Do.	Pinkish; *b* pinkish B.C.; 525–2426.
Black speck...	223	110	49.3	Oct. 16, 1913	Sturtevant.	Zeit. f. I. A. u. V. '15, p. 247, *b c* s_p B.C.
	462	216	46.8	May —, 1914	Muller.....	Am. Nat. '16, p. 422.
	685	326	47.6			
Black balloon.	1,774	857	48.3	Mar. 29, 1913	Sturtevant.	Zeit. f. I. A. u. V. '15, p. 276; B.C.
	462	216	46.8	May —, 1914	Muller	
	2,236	1,073	48.1			
Black morula.	755	353	46.1	Sept. 28, 1913	Bridges....	m_7; *b* m_7 B.C.; II 93–II 96.
	6,794	3,165	46.6	Aug. 4, 1914	Do.	m_7; *b a* m_7 balanced B.C.; 364.
	7,549	3,518	46.6			

The zero position in the second chromosome has been delegated to star, which is found to be 46.5 units to the left of black, that is, black is at an approximate position of 46.5 on the map as recast with star as the zero-point. Table 15 gives a summary of all the cross-over data previously published as well as that given in other sections of this paper.

"BROWN" BODY-COLOR.

Soon after the discovery of black, there appeared in the black stock a few males whose color is a rich "brown" instead of being a clear, cold, greenish black. These flies were in reality a double recessive, as was shown by the F_2 results from the out-crossing of these males to wild females. The other recessive turned out to be "yellow," which is sex-linked. An astounding number of flies (hundreds of thousands) were raised (by Morgan, Wallace, Bridges, and Eleth Cattell) in working out the simple relation of brown to black, to yellow, and to the wild form. A similar interest was shown in the relations of vermilion and pink (the double recessive being called "orange"), these relations being then regarded as highly important from the standpoint of the presence-and-absence theory and the seriation of characters.

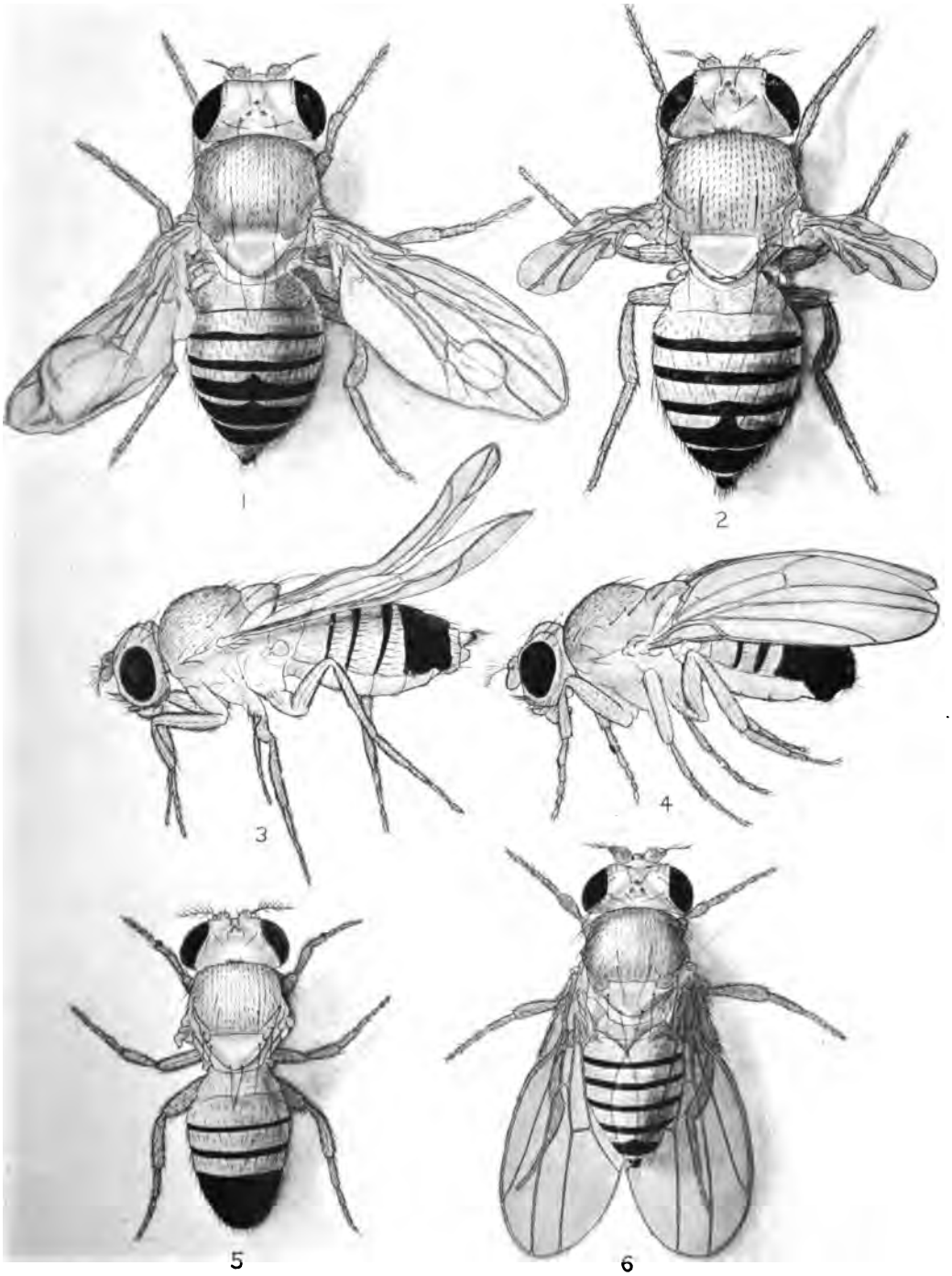
VALUATION.

Black is a mutation of first rank in value, and has been used more extensively than any other autosomal character. Its viability is excellent. With a little practice black can be separated from the heterozygous form with perfect accuracy. There are no other body-color mutations in the second-chromosome that interfere with the classification of black, and in turn black can be used in experiments with any of them (including speck and streak) without masking effects or confusion. Black has been extensively used in class work in genetics by beginners; in this case the only caution necessary is in the classification of very young flies, since the full black color is slow to develop. Even experienced workers occasionally put back into the culture-bottle the very young flies (in a cornucopia, if etherized), and then classify them when they are again taken out at the next counting.

BALLOON (*b*$_2$).

(Plate 7, figure 1.)

The mutant character balloon was found by Morgan (November 1910) in a stock culture of truncate flies (Morgan, 1911). This character was first noticed from the fact that certain flies not long emerged from the pupa-case had their wings pumped full of liquid, the two laminæ of the wing being separated, except at the edges, to form a balloon (plate 7, fig. 1). As these flies became older these vesicles usually broke or the liquid was resorbed, so that the laminæ came together, giving an uneven or blistered appearance to the wing. These wings were held out at a wide angle from the body (plate 7, fig. 1) and this character forms the most quickly recognizable mark of identification in the separations. The divergence of the wings serves well for a quick and rough preliminary separation, though a more reliable


1
2
3
4
5
6

character is the presence of extra veins in the wings, which should be looked for in checking up whether any balloon flies with only slight divergence have been passed over in the preliminary separation. These extra veins are most plentiful as a plexus about the posterior cross-vein and also between the marginal and second longitudinal veins (plate 7, fig. 1). The balloon wing is usually considerably smaller than normal and is of a brownish uneven color and of markedly chitinous appearance. The balloon flies run about very actively and take short, quick jumps, but are unable to fly.

Since balloon appeared in truncate stock it partook of the sterility of truncate. By out-crossing and extraction a stock was obtained which was fully fertile and which was free from truncate.

CHROMOSOME OF BALLOON.

That the gene for balloon is in the second chromosome was shown by Sturtevant (April 20, 1912) by means of a cross to curved, which gave in F_2 the 2 : 1 : 1 : 0 ratio typical in *Drosophila* for experiments with genes in different homologues of the same chromosome pair.

LOCUS OF BALLOON.

Sturtevant also found that the locus of balloon is very far away from that for black (black balloon cross-over value 48.3), and is in fact in the right-hand end in the same region with speck. Speck and balloon were found to be so close together that all attempts to synthesize the double recessive, speck balloon, failed. Without this double recessive it was impossible to run a back-cross test which would have told on which side of speck the balloon gene is situated and exactly how far distant.

By the laborious method of testing individually the offspring of females heterozygous for speck and balloon $\left(\frac{s_p\ +}{+\ b_a}\right)$ Muller (1916) found that two[1] individuals out of a total of 462 represented crossing over between speck and balloon. Balloon is therefore about 0.4 unit away from speck, and to the right as was shown by the other second-chromosome characters tested at the same time.

On account of its marked variability, the character balloon has been used by Marshall and Muller (1917) in a study of the question of the contamination of a gene by its allelomorph when the two are present in the heterozygote. By back-crossing in each generation a male heterozygous for balloon, and for certain other characters used as indexes, to a female which has these index characters but is free from balloon, a stock was carried on for some 50 generations (nearly 3 years),

[1] These two cross-overs were inadvertently omitted from the table of page 422 (Muller 1916) and from his summary on page 423.

during which time the balloon gene was constantly maintained in heterozygous condition. If the effect of the not-balloon gene always present in the homologous chromosome were to render the balloon gene less characteristically balloon-producing, then the balloon stock finally extracted from this long-continued heterozygosis should exhibit a lower grade of the balloon character than that shown by the regular stock of balloon which for some 5 years had been kept homozygous by inbreeding. When the average grade of the individuals of a stock freshly extracted from this heterozygous condition was determined and compared with the like grade determined for the homozygous stock, it was found that the difference from normal of the outcrossed type was *not* less than the difference of the inbred stock. A comparison of the standard deviations of these two stocks showed that there had been no increase in variability on account of the continued heterozygosis. These facts together showed that, in an adequately tested case of character variability, contamination of genes was not operative to a detectable degree.

A summary of the linkage data involving balloon is given in table 16.

TABLE 16.—*Summary of data upon linkage of balloon with other second-chromosome loci.*

Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Streak balloon...	462	242	52.3	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
Dachs balloon...	462	231	50.0	May —, 1914	Do.	Do.
Black balloon ...	1,774	857	48.3	Mar. 29, 1913	Sturtevant.	Zeit. f.i. A. u. V., 1915, p. 276, B. C.
	462	216	46.8	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	2,236	1,073	48.1			
Purple balloon...	462	218	47.4	May —, 1914	Muller.....	Am.Nat.,1916, p, 422.
Vestigial balloon.	462	178	38.5	May —, 1914	Do.	Do.
Curved balloon..	462	150	32.5	May —, 1914	Do.	Do.
Speck balloon†..	462	2	0.4	May —, 1914	Do.	Do.

VESTIGIAL (v_g).

(Plate 7, figure 2.)

ORIGIN AND DESCRIPTION OF VESTIGIAL.

The mutant wing-character now called vestigial was found by Morgan (December 1910) in a stock culture of truncate flies (Morgan, 1911). A few flies of both sexes were found which seemed to have tiny scales in place of wings. The size of the vestigial wing in relation to the size of the body, and the characteristic manner in which these wings are held out at right angles to the body instead of lying back above the abdomen, are shown by the figure. The character was at first called "wingless," and this name appeared in the first few publications

describing it (Morgan, 1911; Morgan and Lynch, 1912; Morgan, 1912). The name "vestigial" was adopted when it was found that the "scale" was the remaining basal portion of the normal wing with the venation characteristic of that region (plate 7, fig. 2). The enormous reduction in the size of the wing is mainly due to the trimming away of the terminal and marginal regions of the wing. There is a marked uniformity in the extent of this trimming and in the character of the venation vestiges. Most commonly the wing is trimmed away as far as the anterior cross-vein, which in many specimens follows the new margin. The true marginal vein with its characteristic chætæ is entirely removed. The basal parts of all five longitudinal veins are easily recognizable, and have their normal relationship and junctures with one another. Certain small veins at the base of the wing are represented here as in the normal wing. The vestigial wing is ordinarily held out at right angles to the body, probably because of the relative thickness of the posterior margin of the wing. Sometimes, however, the ends of the wings are bent sharply backward. These wings seem to be cut off in a squarer fashion than the normal vestigial wing. It is not known whether this variation has any hereditary significance. The "balancers" of vestigial flies are affected in a way analogous to the wings. The basal segment is little affected, except that it is slightly shorter and smaller. The second segment is much reduced in size and in apparent complexity. The terminal segment shows the greatest reduction, becoming a barely discernible pip (plate 7, fig. 2) instead of the balloon-like segment which is the largest part of the normal balancer (see plate 7, fig. 1). Another constant feature of vestigial flies is that the two rearmost bristles on the scutellum are separated a little wider than normal and are erect (plate 7, fig. 2) instead of turning backwards (plate 7, fig. 3). Occasionally vestigial wings are somewhat longer than is typical and it is probable that this lengthening is more frequent during hot weather.[1] The vestigial flies are sometimes inactive, but at other times run about very actively, appearing much like ants. Special care has to be taken with experimental cultures involving vestigial to see that the vestigial flies are all shaken out, since they cling fast to the food or paper in the culture bottle and are exceptionally slow and difficult to get out. The viability of vestigial flies is fairly good, very close approaches to expectation being obtainable when pairs are used and food conditions are favorable. The earlier work showed considerable deviations from expectation because of failure to recognize the necessity of these conditions. Vestigial flies tend to hatch two or more days later than the not-vestigial flies, and unless the cultures are run full term will give ratios in which the numbers of vestigial are below expectation.

[1] Since the above was written, Roberts (J. E. Z., 1918) has strikingly confirmed the fact that high temperature favors the production of wings approaching the wild-type in size.

STOCK OF VESTIGIAL.

Since vestigial, like balloon, appeared in the truncate stock, the vestigial flies were often at the same time truncate, though these could not be distinguished by inspection from the simple vestigials. By out-crossing and extraction a stock was obtained which seemed to be free from truncate (as judged by the absence of truncates among the not-vestigial flies of certain F_1 and F_2 cultures). The balloon mutation which had appeared in the truncate stock just before the occurrence of vestigial was even more difficult to eliminate and occasionally cropped out in the early experiments in which vestigial was used.

INHERITANCE OF VESTIGIAL.

In out-crosses to wild the vestigial appeared to be completely recessive. In F_2 the vestigials reappeared in much less than a quarter of the flies, due to the practice of using mass cultures and to the rather poor feeding methods of that time. Reciprocal crosses gave the same results in F_1 and F_2, so that the gene was known to be not sex-linked.

Lutz (1913) made a biometrical study of wing-length and found that the wings of flies heterozygous for vestigial are slightly but actually shorter than the wings of wild flies. Also, the ratio of wing-length to femur-length was less, showing that vestigial is not completely recessive. It is known in other cases also (see Morgan and Bridges, 1913) that characters that to simple inspection are completely recessive really are influencing the character of the heterozygous individuals.

CHROMOSOME CARRYING VESTIGIAL.

At this time the only case of autosomal linkage known in *Drosophila* was the observation by Bridges that no black curved flies had appeared in the F_2 of the cross of black by curved (Bridges and Sturtevant, 1914). Following this, a concerted testing of the linkage relations of all the known autosomal mutations was carried out. One of these tests, made by Sturtevant and by Miss Clara J. Lynch, showed that in the F_2 of the cross of black by vestigial no black vestigial flies appeared. Both of these cases were put down as very close linkage, "complete repulsion," since it was not yet known that there is no crossing-over in the male whereby this result would be obtained, even though the crossing-over in the female were very free. That crossing-over actually had occurred was shown by the results of mating some of the F_2 black by some of the F_2 vestigial flies. In one of these F_3 cultures some black flies occurred, which meant that at least one of the vestigial parents had been heterozygous for black, $\frac{+ \quad v_g}{b \quad v_g}$, the $b\ v_g$

chromosome being a cross-over. By breeding together some of these F_3 blacks, in F_4 black vestigial flies were obtained from which a stock was made that is still running.

LOCUS OF VESTIGIAL.

By aid of this stock of black-vestigial it was possible to make back-cross tests of the amount of crossing-over. When these experiments were carried out by Morgan it was found that there was about 22 per cent of crossing-over in the female, but none whatever in the male (Morgan, 1912). The principle of no-crossing-over in the male, first clearly demonstrated in the above back-cross, has been found to apply to all cases in both the second and third chromosomes of *Drosophila*.

From the early data of Morgan it was known that the two loci, black and vestigial, were about 22 units from each other. Several errors have been found in the data for crossing-over as first presented (Morgan, 1912). These data, as corrected and considerably added to (Morgan, 1914), show that the locus of vestigial is about 18 rather than 22 units from black.

The mapping of vestigial in relation to the other second-chromosome genes was carried out by Bridges (through the use of purple as a secondary base) and by Sturtevant (through use of curved as a base of reference). Relatively large amounts of data involving the relation of vestigial and other second-chromosome mutants was soon secured. The most useful of these determinations have been the various purple-vestigial data, for purple is the base of reference for vestigial. Table 17 gives a summary of this early data and of all that have since become available.

VALUATION OF VESTIGIAL.

Vestigial, while it is not strictly of first rank in usefulness, approaches very close to this standard. In ease and quickness of separation from the wild-type it is unsurpassed. Its position in the chromosome is one which is important and convenient. Enough work has been done using vestigial as material, so that in undertaking fresh work one can count on a sound basis for comparison. Its viability is good under good cultural conditions. The above are the points in its favor; its disadvantages are that it m[illegible] all other wing-characters, so that its use in an experiment mater[illegible] reduces the availability of other wing-characters, some of which, [illegible]as curved, are themselves of first rank. Its viabilty is apt to be p[illegible]nless careful and experienced attention is given to cultural condi[illegible] and its lateness of hatching and the difficulty of getting the ves[illegible] out of the culture bottle also tend to give aberrant ratios to [illegible]wary.

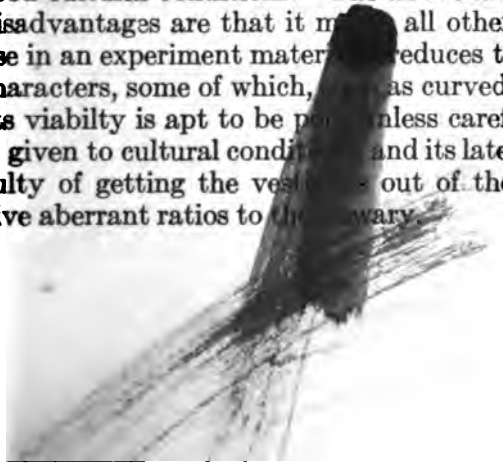


TABLE 17.—*Summary of cross-over data involving vestigial and other second-chromosome loci.*

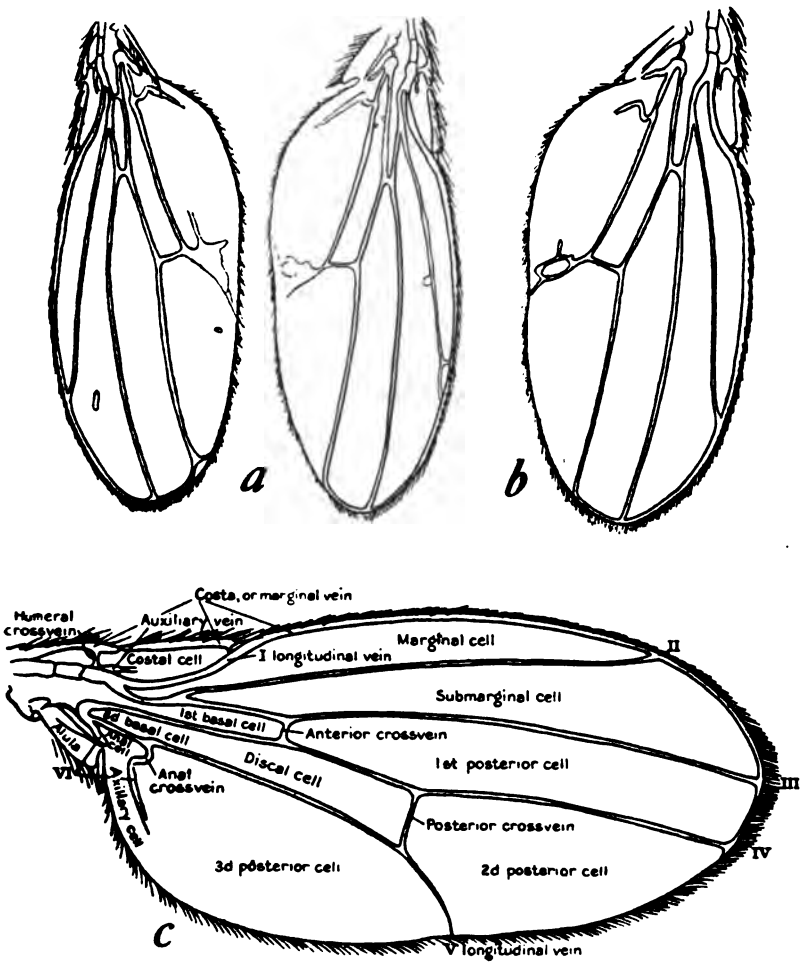
Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Star vestigial..	450	195	43.3	Nov. 17, 1915	Bridges....	v_g^m; $\frac{S'}{b\ v_g^m}$ B.C.; table 123, this paper.
Streak vestigial	462	164	35.5	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
Dachs vestigial	4,892	1,456	29.7	Dec. 10, 1913	Bridges....	*a; d b* v_g balanced B.C.; II 114-II 138.
	462	129	27.9	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	5,354	1,585	29.6			
Black vestigial	3,499	632	18.1	Sept. 10, 1912	Morgan ...	Biol. Bul., 1914, p. 197; $\frac{b}{v_g}$ B.C.
	1,268	217	17.1	Sept. 10, 1912	....Do....	Biol. Bull., 1914, p. 198; *b* v_g B.C.
	694	169	24.4	June 8, 1913	Sturtevant.	Zeit. f. i. A. u. V., 1915, p. 236, *b* v_g *c* B.C.
	4,892	806	16.5	Dec. 10, 1913	Bridges....	*d; d b vg* balanced B.C.; II 114-II 138.
	5,001	815	16.3	Jan. 9, 1914	Muller.....	Am. Nat., 1916, p. 422.
	450	99	22.0	Nov. 17, 1915	Bridges....	v_g^m; $\frac{S'}{b\ v_g^m}$ B.C.; table 123, this paper.
	2,139	477	22.3	Mar. 5, 1917	Plough....	J. E. Z., 1917; *b* p_r v_g B.C.
	1,748	285	16.3	May —, 1917	Gostenhofer	$\frac{b}{v_g}$ B.C.; students records.
	20,153	3,578	17.8			
Purple vestigial	825	79	9.1	July 16, 1912	Bridges....	p_r; p_r v_g B.C.; B 36.1–B 39.2.
	2,839	305	10.7	July 5, 1913	....Do....	p_r; p_r v_g B.C. 1st; DA–DH.
	2,335	303	13.0	July 5, 1913	....Do....	pr; $\frac{p_r}{v_g}$ B.C. 1st; DI–DO.
	5,001	539	10.8	Jan. 9, 1914	....Do....	p_r; *b* p_r v_g balanced B.C., II 141–674.
	462	60	13.0	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	2,139	323	15.1	Mar. 5, 1917	Plough....	J. E. Z.; 1917, *b* p_r v_g B.C.
	13,601	1,609	11.8			
Vestigial curved	856	75	8.8	Mar. 14, 1913	Sturtevant.	Zeit. f. i. A. u. V., 1915, p. 245, v_g *c* B.C.
	402	32	8.0	June 8, 1913	....Do....	Zeit f. i. A. u. V., 1915, p. 247, v_g *c* s_g B.C.
	462	34	7.4	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	1,720	141	8.2			
Vestigial speck	1,446	520	36.0	Mar. 14, 1913	Sturtevant.	Zeit. f. i. A. u. V., 1915, p. 245.
	146	40	27.4	Sept. —, 1914	....Do....	Zeit. f. i. A. u. V., 1915, p. 287.
	462	178	38.5	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	2,054	738	35.9			

BLISTERED (*b*).

(Text-figure 74.)

ORIGIN AND DESCRIPTION OF BLISTERED.

The mutation "blistered"[1] was found by Bridges (November 16, 1911) in a mixed stock of rudimentary and normal (culture A23) as a



TEXT-FIGURE 74.—Blistered wing and venation. 74*a* shows a pair of wings with the bend in the fourth longitudinal vein, and extra veins. 74*b* shows the plexus near the end of the fifth longitudinal vein. 74*c* shows for comparison a normal wing with the standard terminology for venation and for the cells of the wing. It is probable that 74*c* is on a larger scale than 74*a* and 74*b*, though the blistered wing is characteristically smaller than normal.

not-rudimentary female which had in each wing a small vesicle in the region of the fifth longitudinal vein just beyond its junction with the posterior cross-vein. This female was bred to a wild-type brother and

[1] Mac Dowell ('15) published bristle counts on this stock under its earlier name of "half-balloon."

gave only wild-type sons and daughters, from which it was concluded that the character was recessive and non-sex-linked. The F_2 generation gave only a few blistered (about 1 in 10), and these were nearly all females. By mating together the blistered individuals in pairs a stock was obtained which must have been genetically homozygous for blistered, although only about half of the females and about a quarter of the males showed the character. It had been noticed that the size of the vesicle varied from a very minute bubble to one which covered over half the area of the wing, and that there was not a very close correspondence between the two wings; frequently the blister would appear in only one of the two wings. A closer inspection showed that the wings which did not show a vesicle had a small plexus of veins in the region occupied by the vesicle (figs. 74 *a* and *b*), and it was found that the flies could be quite readily classified by this character, irrespective of whether they showed the blister or not. At the same time the results given by breeding from these abnormally veined flies showed that the venation and the blistering were both products of the same gene. A third manifestation of this gene is a sharp bend in the distal end of the fourth longitudinal vein (shown in text-figure 74,*a*).

TABLE 18.—*P_1 mating, curved ♂ × blistered ♀; F_1 mating, wild-type ♀♀ + wild-type ♂♂.*

Apr. 30, 1912.	Wild-type.	Curved.	Blistered.	Curved blistered.
B 12*a*....	81	18	23	0
B 12*b*....	62	25	27	0
B 12*c*....	58	12	6	0
Total	201	55	56	0

THE CHROMOSOME OF BLISTERED.

Little was done with blistered, aside from getting the pure stock just described, until the discovery that black and curved were in the same chromosome gave a sharp impetus to further study of autosomal linkage. Shortly thereafter (April 3, 1912) blistered was crossed to curved and three F_2 cultures were raised (table 18). No curved-blistered flies were found in the F_2. The numbers given in table 18 represent only the first counts from each of these three cultures, and for this reason the number of wild-type flies, which are the first to hatch, is abnormally high, No further records were made of the F_2 offspring, because of the suspicion that blistered might not be distinguishable in curved flies, and that the absence of the double recessive might be due to inhibition or masking instead of the supposed linkage. Nevertheless, all the F_2 flies were examined in the hope of finding a double

recessive. When none was found the experiment was discontinued, since it was thought that the chances of finding a double were as good in F_2 as in any subsequent generation—an attitude excusable at that time, before the fact of no-crossing-over in the male had yet been discovered.

TABLE 19.—P_1, *blistered* ♀ × *pink* ♂; F_1 *wild-type* ♀ *and* ♂.

Aug. 10, 1913.	Wild type.	Blistered.	Pink.	Pink blistered.
M 52....	157	60	58	25
M 53....	146	45	54	13
Total	303	105	112	38

TABLE 20.—P_1, *blistered* ♀ × *black* ♂.

Oct. 23, 1913.	Wild-type ♀.		Wild-type ♂.		Free-vein ♀.		Free-vein ♂.	
II 99.....	27		24		48		4	
B.C., F_1 free-vein ♂ × black ♀.								
Nov. 23, 1913.	Non-cross-overs.				Cross-overs.			
	Black.		Free-vein.		Black free-vein.		Wild-type.	
	♀	♂	♀	♂	♀	♂	♀	♂
II 111....	86	87	60	16	2	.....	30	70
II 133....	25	20	27	9	.....	1	10	19
II 134....	51	45	33	15	2	.....	18	27
II 146....	17	25	25	24	.....	.....	2	9
Total	179	177	145	64	4	1	60	135
B.C., F_1 free-vein ♀ × black ♂. (Nov. 4, 1913.)								
II 102....	67	74	32	10	21	5	32	63
II 109....	44	59	29	11	34	8	49	56
II 132....	39	62	32	8	16	1	49	61
II 144....	20	16	3	6	6	2	25	25
Total	170	211	96	35	77	16	155	205

The tests on the linkage of blistered were taken up again after the discovery of no-crossing-over in the male had made it most probable that the absence of the double recessive curved blistered was due to the genes being in the same chromosome rather than to a masking effect of curved. At this time (July 1913) it was important that any autosomal mutant be tested as to its linkage with both the second and the third chromosomes, and, indeed, it was by the uniform results of many such tests that convincing proof was secured that the second and third

chromosomes are independent in heredity. Accordingly, blistered was mated to pink as the typical third-chromosome mutant. The F_2 from this cross (table 19) gave a 9 : 3 : 3 : 1 ratio, as expected, which confirmed the view that blistered is not in the third chromosome.

Blistered was also mated to black as a representative of the second chromosome; curved was avoided, since it was planned to continue this line and there still lingered some fear of the masking effect of curved on blistered.

THE SEMI-DOMINANCE OF BLISTERED—FREE-VEIN.

When the F_1 flies from the cross of blistered to black (table 20) began to hatch, it was noticed (October 23, 1913) that nearly two-thirds of the females and a few of the males showed a small section of vein lying free in the third posterior cell and parallel to the fifth longitudinal vein. The length of this extra vein was oftenest about two-thirds the length of the posterior cross-vein, but it varies to a minute dot. Flies often showed it in only one wing. This character, while it is very irregular in occurrence, is very easy to classify, since the vein is clear and sharp. The work done with this character is therefore exact and accurate, without the approximations and close decisions that are required in working, for example, with variable colors of slight average difference. It was guessed that this free vein occurring in F_1 was due to the action of the blistered gene, which thus shows an irregular and partial dominance. If this were true it should be possible to work with blistered as a dominant, though only those flies which actually show the character can be used in calculations; many of the others, while somatically normal, belong genetically with those showing the free vein. Accordingly, the F_1 males with the free vein were back-crossed to black females, with the expectation that if the gene for the free vein (blistered) were in the second chromosome all of the offspring would be either black or free-veined, there being no crossing-over in the male. The result showed that the gene was in the second chromosome (table 20), for the flies which were extra-veined were nearly all not-black. There were 5 black flies which showed a slight development of an extra vein, but these veins were probably due to other causes, since 2 of the flies when tested gave 81 and 93 offspring, respectively, none of which showed a trace of the free-vein character. While all of the not-black flies must have been of the same constitution—*i. e.*, heterozygous for the free-vein gene—only 53 per cent of them showed the extra vein.

At the same time that the F_1 free-veined males were back-crossed, some of the similar females were likewise back-crossed (table 20). Since in the female there is crossing-over, this experiment should show the amount of crossing-over between black, whose position is well known, and the gene for the free-vein; that is, we should obtain one of

the two distances required to determine approximately the position of the gene for free-vein. As in the previous experiment, only those flies which actually show the free-vein (about a quarter of the whole number) can be used in the calculation. As shown in the last section of table 20, there were 224 flies with the free vein, of which 93 or 41.5 per cent were cross-overs. When double crossing-over is taken into account, this value indicates that the gene is very far away from black in the second chromosome, probably 50 or more units away. At this time it was known that the dominant mutant streak was in the left end

TABLE 21.—P_1, *free-vein* ♂ × *streak* ♀; *B.C.*, F_1 *streak free-vein* ♀ × *wild* ♂.

Feb. 23, 1914.	Non-cross-overs.		Cross-overs.	
	Streak.	Free-vein.	Streak free-vein.	Wild-type.
69	19 ♀. 20 ♂.	5 ♀. 1 ♂.	4 ♀. 1 ♂.	15 ♀. 16 ♂.

TABLE 22—P_1, *free-vein* ♀ × *speck* ♂.

Feb. 9, 1914.	Wild-type ♀.	Wild-type ♂.	Free-vein ♀.	Free-vein ♂.
19	42	37	14	7
20	18	27	15	13
Total	60	64	29	20

B.C., F_1 free-vein ♀ × speck ♂.

Feb. 27, 1914.	Non-cross-overs.				Cross-overs.			
	Free-vein.		Speck.		Free-vein speck.		Wild-type.	
72	12	9	46	42	1	1	39	32
73	10	2	41	31	1	.....	26	28
Total	22	11	87	73	2	1	65	60

of the chromosome, and while its position was not accurately known, it was at least 30 units to the left of black. The right end of the chromosome was well mapped for at least 50 units, so that while the gene for the free vein might possibly be in either end, it was more probably in the right end. Both of these possibilities were tested. A free-veined male was mated to a streak female, and one of the F_1 streak free-veined females was out-crossed to a wild male, which corresponds in this case to the double recessive. Five of the eleven free-veined flies were streaked (table 21), which free crossing-over means that the free-vein gene is not in the left end of the chromosome and is therefore in the right end. This fact was shown directly by the test with speck, whose locus is in the right end of the chromosome. A free-veined

female (from II 144, table 20) was outcrossed to a speck male, with the regular result shown in table 22. Two of the F_1 free-veined females were back-crossed to speck males (table 22). There were 36 free-veined flies, of which only 3 or 8.3 per cent were speck, *i. e.*, cross-overs. Since black gives about 48 per cent of apparent crossing-over with speck and only 41.5 with free-vein, it is probable that the gene for free-vein lies to the left of speck rather than to the right. Certain more recent work makes it probable that the locus of blistered is considerably nearer to speck, perhaps only two units away, or at 103 ±.

JAUNTY (*j*).

(Plate 7, figure 3.)

DISCOVERY AND STOCK OF JAUNTY.

Shortly after the discovery of "blistered," the wing mutation "jaunty" was found by Bridges (December 11, 1911) in the same stock, rudimentary, in which blistered had appeared. On account of the low productivity of rudimentary females, the rudimentary stock was being carried on by continually back-crossing rudimentary males to heterozygous females. When this method is used, only half of the flies in each generation are expected to show the character rudimentary, the other half being wild-type. The first jaunty seen was one of the not-rudimentary females (notebook A, p. 34) whose wings turned up "jauntily" at their tips. Next day a jaunty male appeared in the same culture. The fact that the character had appeared in both sexes suggested that it was not sex-linked unless dominant. These two jaunties were bred to wild-type flies of the same culture and in F_1 gave offspring none of which showed a trace of jauntiness. That is, jaunty was recessive and autosomal (not sex-linked). Jaunty reappeared in F_2 as approximately a quarter of the flies of both sexes, but no accurate counts were made because the presence of another wing-character, rudimentary, tended to make the classification difficult. To mate mutants to flies of the stock in which they first appear is poor policy because of this presence in succeeding generations of characters which are more apt to be hindrances than helps. In this case it required continued selection of the jaunty not-rudimentary individuals to establish a stock that was pure for jaunty and entirely free from rudimentary.

DESCRIPTION OF JAUNTY.

The wings of jaunty flies are turned upward at their tips, the curvature usually involving only the terminal third or half of the wing, though sometimes the basal region is also more or less curved. The amount of curvature is rarely great enough so that the tip is at right angles to its normal position, the usual curvature being through 30 to

70 degrees. It is this typical curvature of the wing that is used in classification, though, as is usually the case with mutations, there are present various associated or accessory characteristics which are of value, but which in this case must not be relied on over much. Thus, the upper lamina of the wing is frequently corrugated transversely, which is probably the cause of the curvature, though it may be the result. The "inner" side of the wing (posterior margin) is often more strongly curved than the "outer," and in these cases the tips of the wing have a slight oblique slant "outward." The jaunty wings have a tendency to be spread farther apart than normal, though the amount of divergence is slight. In color the wings are a clear gray, slightly darker than normal, and are of strong, thick texture, not flimsy, as in certain other wing mutations. The body-color also is probably a trifle darker than normal. All of these accessory characters are so slight that they would ordinarily be unnoticed.

A "MUTATING PERIOD" FOR JAUNTY.

Soon after the discovery of jaunty, a character which seemed to be the same as jaunty was found (May 1912) by Morgan to be present in the F_2 of a cross of yellow abnormal to white. In rapid succession (May, June, July, 1912) jaunty or jaunty-like characters were discovered in some half dozen stocks or experiments, so that the idea of a "mutating period" for jaunty became current. It is possible that the jaunty mutation did occur on more than one occasion; certainly we were unable to trace any recent or probable connection between three of these appearances of jaunty, namely, the two just given and a case which appeared in the crosses by Bridges of purple vestigial to wild. There are plenty of proved cases in which a mutant character (or an undistinguished allelomorph) has appeared on a second or third occasion, and there is no *a priori* reason why these several mutations might not occur at about the same time. But there are several points to be guarded against before accepting any supposed recurrence of a mutation as genuine. For example, it is surprising through how many generations a recessive will persist in a mass stock even when the character is poorly viable and is selected against in a rough fashion. Thus, the wing mutation "spread" occasionally crops out in the black-plexus stock where it has been carried along "under the surface" for three years, some 75 generations. Any cross made with the black-plexus stock is liable to transmit the spread also, so that a recent case of spread reappearance was finally run to earth as coming from a black-plexus cross made some six months previously. Cases like this are all too frequent and emphasize the value of strict pedigrees and of "cleaned up" stocks. Another point is the pyschological one of recognition. One can fail to see a mutation which has been continually present in flies examined for some generations, and which is so

distinct that when attention has been forcibly drawn to it one wonders how it is possible to have been so blind. Thus, for example, the type "jaunty" having been recognized, suddenly in certain other stocks wings that have been passed over as "imperfectly unfolded" or only vaguely recognized as "queer" are seen to be sharply characterized "jaunties." Again, of the 20 or so mutations previously found it had happened that only 2, rudimentary and truncate, bore much resemblance to each other, and we were then far less critical than now as to whether a new jaunty-like mutation was actually jaunty or only a mimic. To the "genus" jaunty there are now at least four well recognized "species"—jaunty, jaunty X (sex-linked), curled, and California curled. A similar mutating period for purple resulted in isolation of maroon as a distinct but very close mimic and the "mutating period of arc" brought forth arch, bow, arc_2, depressed, and even other types distinct in inheritance though similar in appearance ('arcoids').

TABLE 23.—P_1, *jaunty* ♀ × *black* ♂; F_1 *wild-type* ♀♀ + F_1 *wild-type* ♂♂.

Dec. 16, 1912.	Wild-type.	Black.	Jaunty.	Black jaunty.
II 7.....	546	283	249	0

TABLE 24.—P_1, *jaunty* ♂♂ × *curved* ♀♀; F_1 *wild-type* ♀♀ + F_1 *wild-type* ♂♂.

Dec. 7. 1912.	Wild-type.	Jaunty.	Curved.	Jaunty curved.
C 164....	531	216	285	0

CHROMOSOME AND LOCUS OF JAUNTY.

The fact that the gene for jaunty is carried by the "second" chromosome appeared in the F_2 from the cross of a jaunty female to a black male, when a mass-culture of the F_1 wild-type males and females gave a 2 : 1 : 1 : 0 F_2 ratio (table 23). The F_2 jaunty flies were mated to the F_2 blacks, this being considered the most advantageous method of working for the double recessive black jaunty. In F_3 only wild-type flies appeared. The blacks or jaunties, which would have indicated crossing-over (in the F_1 female) between black and jaunty, and which, indeed, would have given black-jaunty stock in F_4, did not appear. Accordingly these wild-type flies, F_3 offspring, which were like the original F_1, were inbred to give a new F_2, and many more black × jaunty tests were made. Again (F_5) no blacks or jaunties appeared, and this indicated that the loci of black and jaunty are very close together in the chromosome. On several occasions attempts were made by the above method to get the double recessive. By a calculation from the

number of flies tested and proved to be non-cross-overs, it was shown that these loci were probably within 2 or 3 units of each other. Later a more refined method was used, whereby only such flies were tested as were known to be cross-overs very close to black, that is, between dachs and black or between black and purple. The results of these tests only served to bring out more clearly that the locus of jaunty was very close to that of black.

When the black × jaunty cross failed to give the double recessive, a more roundabout method was started by crossing jaunty to curved. The F_2 jaunties (table 24) were inbred instead of being crossed to the F_2 curved. This was to avoid possible doubt as to the double form being separable from curved. Any curved flies descended from the inbred F_2 jaunties must be the double recessive form jaunty curved. Fortunately, jaunty curved flies appeared in F_3 and a stock was made up. The wings of the jaunty curved flies were easily classified as curved, and, surprisingly enough, the jauntiness was usually detectable in the wing-tips. The intended experiments were not carried out with this stock, but Muller (Muller, 1916) used it in building up the multiple heterozygotes which he tested. The tests of Muller furnished 462 flies, of which possibly one was a cross-over between black and jaunty. If this questioned cross-over were genuine, jaunty would be mapped to the right of black and 0.2 unit away. If the apparent cross-over were due to error, then we are only certain that jaunty is exceptionally close to black.

A large series of crosses between jaunty and the third-chromosome mutant pink, carried out by a graduate student, Mrs. Binkley, gave most interesting and puzzling results, the data for which was unfortunately lost with the second-chromosome summaries. As we recall the case, the F_2 ratios were more nearly 4:2:2:1 than 9:3:3:1. The back-crosses likewise gave aberrant ratios which we could not explain as due to viability effects and which were not due to linkage disturbances in the relation of jaunty and pink, since the back-cross tests of F_1 males and F_1 females gave like results, in which the percentage of recombination was approximately 50, as expected. Besides these peculiarities of the ratios there was a curious appearance of white-eyed flies which in inheritance reminded us somewhat of the case of "whiting," an eosin modifier worked out by Bridges, but which was far more elusive in the manner of the alternate appearance and disappearance of white and in the relation of pink to the case.

VALUATION OF JAUNTY.

Though jaunty is easily separable from wild-type and is of good viability and behavior, it is not much used. The main drawback is the closeness of its locus to another locus already satisfactorily filled by black. This region of the chromosome could be represented

equally well by jaunty or by black, but black continues to be chosen, because of its greater prestige, because black already exists in many useful combinations and multiple stocks, while jaunty is not combined with other useful characters, and because black has at present less masking effect than jaunty. The wing-mutations of the second chromosome are relatively numerous and tend to mask each other's effects. Generally, therefore, two wing-characters are not used simultaneoulsy, and the choice falls on the one having the locus best adapted for the particular experiment. If jaunty were in the neighborhood of streak or in any of the long empty regions of the second chromosome it would be considered a mutation of first rank.

CURVED (*c*).

(Text-figure 75.)

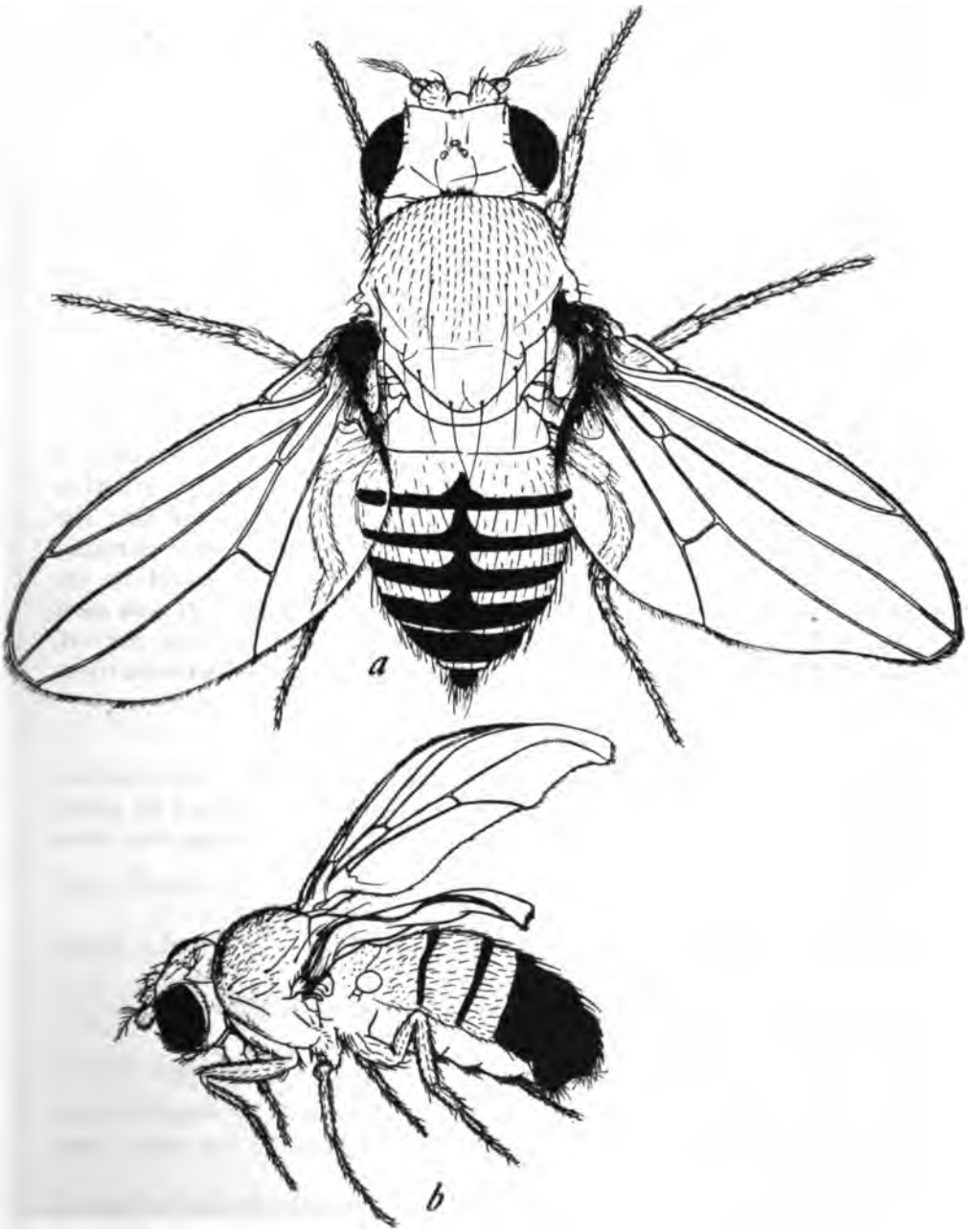
DISCOVERY AND STOCK.

A third mutation in the rudimentary stock, curved wings, was found by Bridges December 24, 1911 (notebook A, p. 43). This mutation appeared in both males and females and both as rudimentary and as not-rudimentary individuals (fig. 75), from which facts it was concluded that the mutation was probably not sex-linked. The not-rudimentary curved males and females were mated together and produced in the next generation a stock about 80 per cent of which was curved; the 20 per cent of not-curved flies was the result of non-virgin mothers.

Virgin not-rudimentary curved females were mated in pairs to similar males, and all of these pairs produced pure stock of curved. Furthermore, about half of the pairs gave no rudimentary sons, and these cultures, known to be entirely free from rudimentary, were used as the permanent stock of the mutation.

DESCRIPTION OF CURVED.

The most characteristic feature of the mutation "curved" is perhaps the thin texture of the wing and its accompanying slight but characteristic crinkliness like waxed paper. More striking than the texture, though perhaps a result of it, is the strong downward curve of the wing. The curved wings are generally held out at a wide angle (60°) from the body and are elevated (30° to 60°). The pose and curvature of the wings are quite bird-like, and the first name of the mutation was "gull." The divergence of the wings, as in all other mutants possessing such a characteristic, is the first index of the mutation to catch the eye when the flies are drawn out in a windrow for separations. In a few of the flies both the elevation and the divergence of the wings are slight, and in these cases the curvature and the texture of the wings are used as indexes. In spite of the thin texture of the wing and the



TEXT-FIGURE 75.—Curved wings. 75*a*, curved as seen from above; 75*b*, as seen from the side and above. (Fig. 75, *b*, shows speck also, having been drawn from a purple curved speck fly.)

wide angle at which it is held, the curved wing seldom becomes bedraggled, and the flies are very free from the tendency to become stuck in the food or to be drowned. As far as we have observed, the wings are the only part affected by the curved mutation.

CHROMOSOME OF CURVED.

The first linkage in *Drosophila* that did not involve the X chromosome was that observed by Bridges (March 1912) between black and curved (Bridges and Sturtevant, 1914). In the F_2 generation of a cross of black to curved no black-curved flies appeared. This case was interpreted as one in which the linkage was of such a strong order that no crossing-over had taken place. On the basis of this linkage it was concluded that curved was in the same chromosome as black, that is, in a "second chromosome." A systematic search for linkage between the other known non-sex-linked genes in *Drosophila* was undertaken, and a similar relation, "repulsion," was studied in the case of black and vestigial (Morgan and Lynch, 1912). Further work by Morgan in the case of black and vestigial showed that the non-appearance of the double recessive, where two second-chromosome recessives entered the F_1 from opposite parents, could be explained on the basis of lack of crossing-over in the male. It was soon shown that this same explanation applied in the case of black curved, and that in the female there is considerable crossing-over between these two loci.

LOCUS OF CURVED.

In order to obtain stock of the double recessive, the black-curved cross was repeated, and some of the F_2 blacks were mated in mass-cultures to the F_2 curved flies of the other sex. If crossing-over were taking place in the F_1 female $\left(\frac{b\ +}{+\ c} \longrightarrow \frac{b}{+} \middle| \frac{c}{+}\right)$, a few of these F_2 blacks should be heterozygous for curved $\left(\frac{b\ +}{b\ c}\right)$ and a corresponding few of the curves should be heterozygous for black $\left(\frac{+\ c}{c\ c}\right)$. The appearance in F_3 of a few black flies $\left(\frac{b\ +}{b\ c}\right)$ showed that at least one of the F_2 curved flies had been the result of crossing-over. By inbreeding these F_3 blacks, black curved flies (25 per cent) were secured in F_4.

That the absence of black curved flies in F_2 was really due to lack of crossing-over in the male was shown by the results of the back-cross tests carried out upon double heterozygous males as compared with like tests of the females. In the tests of the males no cross-overs appeared in a total of 1,066 flies, while in the tests of the females 1,717

TABLE 25.—*Summary of cross-over data involving curved and other second-chromosome loci.*

Loci.	Total.	Cross-overs.	Per-cent.	Date.	By—	Reference.
Star curved...	6,766	3,164	46.8	July 11, 1915	Bridges....	$S'; \frac{S'}{p_r\ c\ s_p}$ B.C. 1sts; 1836–'94.
	13,104	5,959	44.4	Dec. 15, 1915	Plough....	J. E. Z., '17, $\frac{S'}{b\ c}$ B.C. controls.
	19,870	9,123	45.9			
Streak curved..	1,807	745	41.2	Nov. 6, 1913	Bridges....	S'_k; S' balanced B.C.; II 103–124.
	462	178	38.5	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	2,269	923	40.7			
Dachs curved..	462	145	31.4	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
Black curved..	7,419	1,717	23.1	Jan. 13, 1913	B. & Strt..	Biol. Bull., 1914, p. 209; $b\ c$ and $\frac{b}{c}$ B.C.
	260	69	25.5	Jan. 13, 1913	Do.	Biol. Bull., 1914, p. 208; $b\ c$ F_2.
	253	66	26.1	Jan. 13, 1913	Do.	Biol. Bull., 1914, p. 212; $\frac{b}{c}$ B.C.
	402	120	29.9	June 8, 1913	Sturtevant.	Zeit. f. i. A. u. V., '15, p. 247; $b\ v_g\ c$ B.C.
	3,934	839	21.3	Aug. 24, 1913	Bridges....	p_r; $b\ p_r\ c$ B.C. 1sts; II 58–II 88.
	223	63	28.2	Oct. 16, 1913	Sturtevant.	Zeit. f. i. A. u. V.; '15, p. 247; $b\ c\ s_p$ B.C.
	462	106	22.9	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	36,622	8,598	23.4	Jan. 5, 1915	Plough....	J. E. Z. 1917, $b\ p_r\ c$ B.C. controls.
	13,104	2,659	20.3	Dec. 13, 1915	Plough.....	J. E. Z. 1917, $\frac{S'}{b\ c}$ B.C. controls.
	62,679	14,237	22.7			
Purple curved.	3,934	711	18.1	Aug. 24, 1913	Bridges....	p_r; $b\ p_r\ c$ B.C. 1sts; II 58–II88.
	1,807	375	20.7	Nov. 6, 1913	Do.	S'_k; $S'_k\ p_r\ c$ balanced B.C.
	462	90	19.5	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	952	182	19.1	Aug. 24, 1914	Bridges....	s_p; $p_r\ c\ s_p$ B.C.; 452–508.
	36,622	7,222	19.7	Jan. 8, 1915	Plough....	J. E. Z. '17, $b\ p_r\ c$ B.C. controls.
	6,766	1,508	22.3	July 11, 1915	Bridges....	$S'; \frac{S'}{p_r\ c\ s_p}$ B.C. 1sts; 1836–'94.
	593	117	19.7	Feb. 8, 1916	Do.	l_{IIa}; $b\ p_r\ c$ F_2; 3203–'08.
	51,136	10,205	19.9			
Lethal IIa curved.....	249	22	8.7	Dec. 21, 1915	Bridges....	l_{IIa}; $l_{IIa}\ c$ F_2; 2675, 2840; '59, '60, '63.
Vestigial curved.	856	75	8.8	Mar. 14, 1913	Sturtevant.	Zeit. f. i. A. u. V., p. 245, $v_g\ c$ B.C.
	402	32	8.0	June 8, 1913	Do.	Zeit. f. i. A. u. V., p. 247, $v_g\ c\ s_p$ B.C.
	462	34	7.4	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	1,720	141	8.2			
Curved speck..	1,007	262	26.0	Mar. 10, 1913	Sturtevant.	Zeit. f. i. A. u. V., '15, p. 245; $c\ s_p$ B.C.
	223	71	31.8	Oct. 16, 1913	Do.	Zeit. f. i. A. u. V., '15, p. 247; $b\ c\ s_p$ B.C.
	462	150	32.5	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	952	266	27.9	Aug. 24, 1914	Bridges....	s_p; $p_r\ c\ s_p$ B.C.; 452–508.
	6,766	2,062	30.5	July 11, 1915	Do.	$S'; \frac{S'}{p_r\ c\ s_p}$ B.C., 1sts.; 1836–'94.
	632	226	35.7	Feb. 8, 1916	Do.	l_{IIa}; $p_r\ c\ s_p$ F_2; 3203–'08.
	10,042	3,037	30.2			
Curved balloon	462	150	32.5	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.

cross-overs appeared in a total of 7,419 flies (Bridges and Sturtevant, 1914). In the male there was no crossing-over whatever, while in the female there was about 25 per cent.

The direction along the II chromosome from black to curved was called "to the right," and curved was therefore mapped at a locus 25 units to the right of black.

The position of curved has been more accurately determined by use of intermediate loci than was possible from the rather long black curved interval. Thus the purple curved stock, made by Bridges in preparation for the triple back-cross black purple curved, was used by Mr. W. S. Adkins to run extensive purple curved back-cross tests. These crosses, the data for which are not yet available, gave about 18 per cent of crossing-over between purple and curved, which agrees with the result of the black purple curved back-crosses. Much data has since been collected on this cross-over value, largely incidental to the work on age variation by Bridges and temperature variation by Plough. A total of 51,136 flies included 10,205 cross-overs between purple and curved; the observed percentage of crossing-over was thus 19.9. It is possible that there is considerable double crossing-over within this region, since it is in the middle of the chromosome, where double crossing-over in relation to map-distance has been found to be extraordinarily high. If a coincidence of 70 is assumed, the corrected purple-curved value becomes 21.4 and the locus of purple is 27.6 units to the right of black.

The locus of curved was also referred to vestigial by Sturtevant, who ran vestigial curved and black vestigial curved back-crosses. This method has great advantages in mapping the locus of curved, since vestigial is itself accurately mapped in relation to purple and is an intermediate base between purple and curved. Only the not-vestigial back-cross flies can be used in the calculation, since curved vestigials can not be distinguished from the simple vestigial class. Hence the vestigial curved data are too meager as yet (1,720 flies) to be used as the main basis of the location of curved.

A summary of the cross-over data involving curved and other second-chromosome loci is given in table 25.

VALUATION OF CURVED.

Curved is in all respects a mutant character of first rank, both for student use and in special experiments. Its separability from the wild-type is both easy and accurate, even without experience. Its viability is excellent. It causes no trouble through liability to drowning or miring, which might have been expected on account of the strongly divergent wings. Its locus is the outpost of the central body of well-mapped genes and it is therefore the base of reference for speck and for all genes near the right end of the chromosome.

PURPLE (p_r).

(Plate 5, figure 8.)

ORIGIN OF PURPLE.

In a stock kept in the stock-room and supposed to be simply vestigial, there was found, February 20, 1912, a single male which had an eye-color much like that of the well-known double recessive vermilion pink. The color of the vermilion pink eye is about that of the pulp of an orange, and the early papers accordingly referred to this double recessive as "orange." The new color was seen to differ slightly from vermilion pink in that it was of a brilliant ruby-like transparency and lacked the flocculent or slightly cloudy appearance of vermilion pink. This difference seems to arise partly from a difference in the distribution of the pigment. In vermilion pink the pigment looks as though it were mainly in the spaces between the radially arranged ommatidia with a clearer zone just under the surface of the eye. One sees in the vermilion pink eye a light fleck which travels over the eye as it is turned. This seems to be due to a deficiency of pigment in the deeper parts of the eye and the light fleck is this light center seen through the small group of facets whose axes are in line with our eye. The pigment in the case of the new eye-color gave the appearance one would expect if it were uniformly distributed or even in solution throughout the eye.

INHERITANCE OF PURPLE.

This single male with the orange-like eye-color was out-crossed to a wild female, and in F_1 gave only wild-type males and females (wild-type ♀ 32, ♂ 33; reference No., B 1) which showed that the color was recessive. In F_2 the orange-like color reappeared, but in addition the sex-linked eye-color vermilion emerged, and also a new eye-color "purple" which appeared equally among the F_2 females and males and was therefore known to be an autosomal (not sex-linked) character. It was now evident that the orange-like color resembled the old "orange" (vermilion pink) genetically as well as somatically, for it was proved by this F_2 to be a double recessive, vermilion purple, in which purple corresponds to pink.

It seems probable that the two eye-color mutations, vermilion and purple, present in the male first found were not of simultaneous or related origin. There was a vague rumor that the vestigial stock had contained vermilion at some time previous to this discovery. No vermilion or purple was found in it subsequently, however.

DESCRIPTION OF PURPLE.

The eye-color of purple flies passes, in its development, through an interesting cycle of changes closely parallel to those seen in the ripening of a sweet cherry. In the pupa the eye is at first colorless, then it

assumes a creamy tone which in turn becomes pinkish, passing progressively through a yellowish pink to pink and to ruby. When the flies hatch, the color is a transparent rather deep ruby. This color rapidly deepens to garnet and then passes on to a purplish tone. The typical purple color at its maximum development—in flies about a day old—while retaining much of its transparency, appears darker in tone than the red of the wild-type, purple being the first of such "dark" eye-colors. As the fly becomes older this "ripe-cherry" color is progressively obscured, apparently by an increase in a flocculent red pigment, like that of the wild fly. The eye-color thus becomes somewhat lighter than red again, though always distinguishable by a lesser opacity and by a light "fleck" in place of the hard dark fleck seen in the wild eye. With extreme old age the color approaches still closer to red, but does not become strikingly darker, as do pink and sepia, for example. In purples of the same age fluctuations in color are not great. Separations are easy if done, as usual, while the flies are mostly under 2 days old, though the climax in the development of the purplish tone offers the most favorable stage.

THE DIFFERENTIATION OF PURPLE BY VERMILION—DISPROPORTIONATE MODIFICATION.

While the difference between the color produced by the purple gene and the color produced by its wild-type allelomorph (red) is distinct, it is neither great nor striking, since in tone purple is first slightly darker and later somewhat lighter than red. However, in classifying the eye-colors in F_2 from the cross of vermilion by wild, it was observed that the difference between vermilion purple and vermilion not-purple was not only constant in direction, but also conspicuous in extent. The separability of purple *versus* not-purple is favored by the presence of vermilion, which may therefore be called a "differentiator" of purple. Regarded in the converse relation, namely, the effect of purple on vermilion rather than the effect of vermilion on purple, purple is a much stronger modifier of vermilion than of not-vermilion. Purple may be described as a "disproportionate modifier" of vermilion, since from the small amount of its effect on eye-color when acting alone one would not have expected the great effect it produces when acting in the presence of vermilion.

This type of intensification—disproportionate modifier and, conversely, differentiator—stands midway between the normal relation where combination effects are roughly proportional to the separate effects, so that both genes may be called "general modifiers," and the special relation where a given gene, "specific modifier," produces by itself no visible effect whatever, but which gives a more or less marked effect when acting in conjunction with some other gene, its specific base, sensitizer, or differentiator.

In order to make full use of this differentiation of purple *versus* not-purple by vermilion, it is necessary that all flies used in the experiment should be made homozygous for vermilion. This is often inconvenient, and accordingly only in the early and comparatively simple experiments was this method employed. It was soon found also that the separation of purple from red was not causing any trouble, so that the differentiation in this case has little net advantage, though it is still of interest as being the first example in *Drosophila* in which intensification was recognized and deliberately made use of.

THE RELATION OF PURPLE TO PINK.

Some of the first purples which emerged in the F_2 were crossed to pink to test whether these two eye-colors were allelomorphic or not. Only wild-type F_1 males and females were produced (table 26), which showed that the purple is not an allelomorph of pink.

TABLE 26.—*F_1 progeny from out-cross of purple.*

Apr. 17, 1912.	Wild-type ♀.	Wild-type ♂.
B 3.1....	33	31
B 3.2....	27	25
B 3.3....	23	28
B 4.....	54	50
Total	137	134

THE LINKAGE OF PURPLE AND VESTIGIAL.

It was observed (April 2, 1912; B1) that in the F_2 from the cross of the original male to wild nearly all of the flies that were purple were also vestigial. This observation, following on the heels of the black-curved case, furnished a second example of autosomal linkage, this time one of so-called "coupling," the black curved case having been "repulsion." No full counts were made of the proportion of purples that were vestigial. Indeed, at this early stage the linkage relations were receiving less attention than eye-color "series."

BACK-CROSS TEST OF MALES, PURPLE VESTIGIAL "COUPLING."

The advantages of the back-cross method of testing linkage and the amount of crossing-over had only begun to be appreciated. This method had been applied to a few cases in the X chromosome, and the general attack upon the linkage of all autosomal mutations planned by Sturtevant and Bridges (March 5, 1912) contemplated its full use. Thus far only two autosomal back-crosses had been completed—those by which Sturtevant showed the absence of linkage between the second chromosome and the third chromosome (balloon ebony, May 10, 1912, and black pink, May 12, 1912). Because of the diffi-

culty of getting the necessary double recessives no back-cross which involved autosomal linkage had been possible until purple arose in the vestigial stock and thereby gave the required double recessive, purple vestigial, with which such a test of the amount of crossing-over between purple and vestigial could be conducted. From the F_2 described above, matings were made which gave two stocks to be used in this test. One stock was the simple purple vestigial and the other was purple vestigial pure for vermilion. The special advantage of this latter stock lay in the fact that the presence of vermilion accentuates the difference in eye-color between the flies that are purple and those that are not; that is, vermilion purple is easier to separate from vermilion than is the case in the equivalent separation of purple from red.

TABLE 27.—*B. C. offspring given by the F_1 (vermilion) sons, from out-cross of (vermilion) purple vestigial males to vermilion females, when back-crossed to (vermilion) purple vestigial females.*

June 24, 1912.	Non-cross-overs.		Cross-overs.	
	(Vermilion) purple vestigial.	(Vermilion.)	(Vermilion) purple.	(Vermilion) vestigial.
B 10.1...	90	186	0	0
	71	202	0	0
B 10.2...	72	197	0	0
	72	206	0	0
B 11.1...	45	126	0	0
	65	195	0	0
B 11.2...	51	88	7	3
	98	178	27	2
	43	72	4	0
B 11.3...	54	191	0	0
	37	70	0	0
Total	698	1,711	38	5

This latter stock was accordingly used in the P_1 mating for the first back-cross test. Vermilion purple vestigial males were out-crossed to females of vermilion stock (May 25, 1912). Both parents were homozygous for vermilion, and the F_1 flies were all vermilion, as expected. Both purple and vestigial are recessive. When the back-cross matings came to be made the culture bottle happened to contain no virgin F_1 females, since the P_1 mating had been made at Columbia and the F_1 progeny used had hatched en route to Wood's Hole. The back-cross was therefore made in only one way—by mating the F_1 *males* to virgin vermilion purple vestigial females of the stock kept for that purpose. Five back-crosses were started by mating in each case a single F_1 vermilion male by two or three stock vermilion purple vestigial females. At the end of 10 days the parents were removed from the culture bottles and put in fresh bottles in which second broods were raised. In one case a third brood was raised (table 27).

The linkage results of these back-crosses were somewhat unexpected, for in four of the lines no cross-overs at all were obtained, and in another only a few. In the original F_2 culture several cross-overs had been noted, and five F_2 cultures raised from the brothers and sisters of these back-crossed males seemed to be giving in the neighborhood of 15 per cent of cross-overs (table 28). A suspicion was aroused that the results of the back-cross and of the F_2 were of a different order, but this idea did not develop beyond a suspicion because of the contradictory result given by the different back-cross cultures. It was not at first clearly realized that these aberrant cultures were descended from a single set of parents, so that the difference was disproportionately blurred. Whatever difference was recognized was attributed to a possible difference in the linkage results given by back-crosses as contrasted with F_2's. Since this was the first back-cross involving autosomal linkage that had been tried, there was no corrective evidence to show that these two kinds of results might not be different.

TABLE 28.—*F_2 offspring given by the F_1 (vermilion) sons and daughters from the out-cross of (vermilion) purple vestigial males to vermilion females.*

June 17, 1912.	(Vermilion).	(Vermilion) purple vestigial.	(Vermilion) purple.	(Vermilion) vestigial.
B 8.1....	200	23	9	5
B 8.2....	88	21	3	4
B 8.3....	255	66	25	5
B 9.1....	368	19	3	7
B 9.2....	346	17	19	9
Total	1,257	146	59	30

BACK-CROSS TEST OF FEMALES, PURPLE VESTIGIAL "COUPLING."

A second back-cross experiment using the simple purple vestigial stock instead of the vermilion purple vestigial was started (June 25, 1912) a month later than the first and before the results of the first were fully known. A purple vestigial male out-crossed to a wild female produced wild-type sons and daughters (B 39; + ♀ 15, + ♂ 10). Four of the F_1 females were back-crossed each by two or three purple-vestigial males from stock. In this case F_1 females happened to be chosen because, as is usually the case, they hatched somewhat earlier than their brothers in the same culture.

These back-cross cultures (table 29), in common with the previous F_2 cultures (table 28), showed a fair amount of crossing-over between purple and vestigial. A calculation showed that the percentage of crossing-over was 9.1. This was recognized as being of a different degree from the apparent percentage of 1.8 calculated from the first back-cross (table 27). It was now realized for the first time that the two back-crosses had differed in the sex of the F_1 flies tested by the

back-cross—that the first back-cross was a test of the amount of crossing-over in the male and the second was of crossing-over in females. Up to this time there had been no suspicion that the result of a back-cross could be in any way dependent on the sex of the F_1 parent used in the experiment. From this evidence it was concluded that there was crossing-over in the male, but that it was of different degree from that in the female. In September 1912, Morgan showed that in the case of black vestigial no crossing-over whatever had

TABLE 29.—*B. C. offspring given by F_1 daughters, from the out-cross of a purple vestigial male to a wild female, when back-crossed to purple vestigial males.*

July 16, 1912.	Non-cross-overs.		Cross-overs.	
	Purple vestigial.	Wild-type.	Purple.	Vestigial.
B 36.1...	82	163	12	15
B 36.2...	80	133	14	10
B 39.1...	32	53	3	7
B 39.2...	62	141	9	9
Total	256	490	38	41

occurred in the male, while in the female there was even more crossing-over than had been found in the case of purple-vestigial. Subsequent tests, including hundreds of thousands of individuals, have shown that ordinarily there is no crossing-over in the male for any chromosome and that the few (2) cases that have occurred were probably not brought about by the same mechanism as that by which crossing-over is ordinarily effected.

NO CROSSING-OVER IN THE MALE.

A clear conception of the fact of no crossing-over in the male was obscured in the original vermilion purple vestigial back-cross test by the apparent occurrence of cross-overs in one of the five cultures. It is still a matter of doubt as to what actually occurred in that culture. No tests were made of the apparent cross-overs, because there was at that time no evidence, aside from the inconsistency within the experiment, to suggest that they were very unusual. It is strongly suggestive of error that in this first male test, carried out before we were on guard, so many apparent cross-overs should have occurred, and that in the numerous and extensive tests made subsequently they should be so strikingly absent. Perhaps some clerical error was committed—such, for example, as a mistake in labeling—that this culture was really one of the F_2 cultures that had been made up from the same F_1 culture, though at a different time from the back-crosses.

At that period, it is true, methods of keeping records were poor as compared with present standards, and errors were all too frequent. Against the supposition that this particular mistake was made is the internal evidence that the proportion of vermilion purple vestigials in the questioned culture resembled that in the other back-cross cultures and is larger than that in any of the rightly labeled F_2 cultures. Again, that this culture should be a back-cross test of the female rather than of the male would require a double error—*i.e.*, as to the sex of both parents—and this error would probably have been detected at the time of the transference of the parents to fresh culture-bottles, especially since these parents were transferred to a third culture-bottle. The suggestion has been made that "maroon," a third-chromosome recessive eye-color resembling purple very closely, had been present, probably only in heterozygous form, in the vermilion purple vestigial stock, and that the introduction of maroon through the vermilion-purple vestigial parent at the P_1 and again at the back-cross mating would account for the cross-over class taken to be vermilion purple in the progeny. Such an explanation fails to account for the complementary class of exceptions, the few but carefully attested vestigials that were not-purple. Several other suggestions have been made, and while it seems highly probable in the light of the more recent work that these apparent cross-overs were really due to error in the conduction of the experiment or to unknown properties of the stocks used, none of the suggested escapes from the alternative that there really had been crossing-over in this particular F_1 male have solved all the difficulties.

If these were true cross-overs, it is still possible that their production should have no relation to the mechanism by which crossing-over is ordinarily effected. Thus, Muller (1916) reported a case of crossing-over in the back-cross test of a certain F_1 male from the mating of truncate to black. However, all of the gametes of this particular F_1 male proved to be cross-overs, so that crossing-over must have occurred, once for all, in an early cell of the embryo, and, as usual, no crossing-over whatever occurred during spermatogenesis. The spermatozoa, all of which were descended from this embryonic cross-over cell, simply inherited the cross-over combination. In the case of purple vestigial a like explanation would apply, except that in this case the crossing-over occurred in a somewhat later stage of the embryo and in consequence only a part of the spermatogonial cells carried the cross-over combination, and only sperm descended from these particular cells produced cross-over progeny.

That somatic crossing-over has little analogy to the ordinary type is proved by a similar case of embryonic crossing-over in the female, which was followed by crossing-over of the ordinary type. A mating

was made, such that a certain class of daughters should all have the composition $\frac{v \quad l_9 \quad + \quad + \quad B'}{+ \quad + \quad s \quad g \quad +}$. Seven of the eight daughters tested had this expected composition, but one (No. 3464) gave only offspring corresponding to the composition $\frac{v}{+} \Big| \frac{+}{l_9} \Big| \frac{+ \quad + \quad B'}{s \quad g \quad +}$. That is, the gene for lethal 9 was found to be not in the chromosome in which it entered the zygote, but in the homologous chromosome derived from the other parent. As in the truncate × black case, this transmigration took place after fertilization and so early in the embryonic history that all the germ-cells were descended from this altered cell. A significant feature of this case is that while the change must be described superficially as double crossing-over, this double crossing-over occurred within a region only 10 units long—a space shorter than that in which double crossing-over of the ordinary type has ever been detected, even in certain regions of the autosomes in which double crossing-over is relatively frequent.

OTHER MUTATIONS.

Two new mutations were found and two old ones recurred in these back-cross experiments on the linkage of purple and vestigial.

"Kidney" eye-shape, a third-chromosome recessive, was found in B. C. culture B 102, June 26, 1912 (table 27). This mutant, the first affecting the shape or texture of the eye, was considerably used in the early days (see Morgan, 1914, and Bridges, 1915), but has now been superseded by mutants less variable and easier to classify.

In culture B 39.2 it was noted, July 26, 1912, that several wild-type flies had more bristles on the thorax than the regular number, *i. e.*, 4. Later it was found that such extra-bristled flies were occurring in small proportions in all four sister cultures, from which it would appear that the mutation was a recessive, introduced through the purple-vestigial stock used twice in the experiment. The extra bristles occurred among all classes in the experiment indifferently, which would seem to indicate that the gene was not second-chromosome, since if it were the extras should have been relatively more frequent among the purple vestigials. The number of extra bristles varied from 1 to 4, the highest total bristle number observed being 8. Extra bristles were also observed to be frequent in two or three other stocks. A stock throwing extra thoracic bristles derived from B 39.2 was maintained by rough mass selection for some time and was finally given to Mr. E. C. MacDowell to be used as the basis of rigorous selection experiments (MacDowell, 1915). As the result of a survey of all stocks known or suspected to contain extra bristles, MacDowell chose a certain wild stock as the most favorable starting-point for his selection.

In culture B 9 a jaunty (jaunty 4) appeared which gave rise to a stock similar to the original jaunty, but so far as known of separate origin.

In three or four of the cultures, for example in B 9.1, arc wings (arc 6) appeared, and these were indistinguishable from the original arc, though quite certainly of different origin.

Since these early experiments many other mutations have arisen in experiments involving purple, but these need no special mention here.

THE INVIABILITY OF VESTIGIAL—PREMATURATION, REPUGNANCE, LETHALS.

One of the most striking features of these crosses involving purple and vestigial was the failure of vestigial to appear in as high a proportion as expected. In the F_2 (table 28) where 25 per cent of the flies were expected to be vestigial, only 12 per cent were vestigial; in the back-crosses, where half of the flies were expected to be vestigial, only 29 per cent (table 27) and 36 per cent (table 29) were vestigial; that is, only about half as many vestigials as were expected appeared in these back-crosses.

Such a condition is usually described by the blanket term "inviability;" but a consideration of the "inviability" met with in the case of rudimentary (Morgan, 1912) had just led to two new conceptions: first, that the power of fertilization possessed by a gamete is influenced by its somatic environment prior to maturation; second, that a given type of egg is less likely to produce a viable zygote with one than with another of two classes of sperm. The conception of "prematuration" was used to account for the fact that a rudimentary-bearing egg from a pure rudimentary female is much less able to give a viable offspring than a like egg from a mother only heterozygous for rudimentary. The principle of "repugnance" was exemplified by the cross of rudimentary by rudimentary, which gave no offspring whatever, though repeated fully 100 times, and although both the male and female give offspring when out-crossed. The rudimentary females are usually sterile, and never give more than a few offspring (nearly all females) when out-crossed to unrelated males.

The shortage of vestigials in the above crosses was thought to be parallel to the results given by rudimentary, except that in the case of vestigial the effects of prematuration and repugnance were not as great in degree. On the basis of these results, an analysis of the extent to which each of these principles contribute to the "inviability" of vestigial was undertaken by Mr. G. L. Carver (results not yet published). In Mr. Carver's investigation it was assumed that the shortage in these experiments had been largely due to a cause intrinsic in the vestigial itself, for which reason any stock of vestigial should be equally valid for the test. The stocks used in the above experiments

were not used, because they were full of odds and ends of mutants which might lead to confusion. The tests showed that very little prematuration or repugnance is inherent in vestigial, the ratios being exceptionally close to Mendelian expectation; wherefore it seems probable that the shortage met with in the purple vestigial experiments was due to some cause peculiar to the stocks used or to the culture methods used in the experiments. Later tests with stocks descended from these original stocks have failed to give such aberrant viability.

Another explanation that has been more recently applied to particular instances in which a character ordinarily of excellent viability has not appeared in the expected proportion, is that of a lethal gene. Thus, an autosomal lethal in the second chromosome quite far to the right of vestigial (*i. e.*, close to speck) would give results roughly-comparable to those observed. The difficulty with such an explanation in this case is that the uniform results given by all the cultures would require the lethal to be present in nearly all the individuals, a frequency entirely out of the question both from *a priori* considerations and from the results of subsequent tests made with these stocks.

THE PURPLE "EPIDEMIC"—MUTATING PERIODS.

Shortly after the discovery of purple, purples or eye-colors closely resembling purple began to be found in stocks and experiments everywhere. In the interval of 6 months following the discovery of purple such occurrences numbered 14 and furnished the first as well as the most striking of the "epidemics of mutation" that seemed to sweep over our material at this period. From later and well-authenticated cases (*e. g.*, vermilion, cut, notch, etc.) it appears that certain mutations do recur, and in the case of cut, four independent occurrences followed one another so closely that the term "epidemic" is descriptive of the condition observed. However, in the early cases (purple, jaunty, arc, etc.) it is certain that a large majority of the apparent cases were not true recurrences of the mutative change, but were due to several other conditions. Thus, the first, fifth, sixth, and thirteenth of the apparent purples proved to be maroon, a third-chromosome eye-color practically indistinguishable from purple in appearance. That is, "mimic" mutations were not at first distinguished from the original type, nor were new mutant allelomorphs distinguished from types already known unless the difference was striking. Certain others of the occurrences were proved not to be of independent origin; thus, purples 8 and 9 were both shown to have been descended from a certain common stock, and purples 10 and 11 were traced to a second common stock. It is undoubtedly true that in many cases where no connection can be traced such connection really existed, especially in the case of recessives, which might be distributed without giving sign of their presence. The psychological element, too, is important; it is exceedingly difficult to recognize a mutative change, even a striking

one, before one becomes "sensitized" to that particular mutation. Some of our mutant characters had long been present in stocks or experiments, so that many flies showing the character must have been seen, before attention become sharply focused upon the differences shown. Contamination and errors of one sort or another have also added to the number of apparent reoccurrences of mutations. It is therefore to be doubted if more then two of the apparent reoccurrences of purple were genuine remutations.

REPETITION OF THE PURPLE VESTIGIAL BACK-CROSS TESTS.

Because of the number of disturbing conditions that had been met with in the first set of tests of the linkage of purple and vestigial, a second and more extensive set was started. These second experiments were carefully planned, and in the results obtained approach present standards of uniformity and reliability. The viability of vestigial was excellent, and the equality of contrary classes throughout the experiments speaks for the favorable culture conditions. The new experiments were conducted with a purple vestigial stock descended from that used in the experiments of table 28, but cleared of mutations and perhaps other disturbing factors by out-crossing to wild and by selection, started among the F_2 progeny and maintained for several generations until it seemed probable that the stock was clean. Also, from the progeny of table 28 some purple (not-vestigial) cross-overs were selected and from them was secured in a few generations a simple purple stock free from vestigial and from the other mutant characters known to be present.

TABLE 30.—*F_2 offspring from cross of a purple male to a wild female.*

Nov. 25, 1912.	Wild-type ♀.	Wild-type ♂.	Purple ♀.	Purple ♂.
C 178....	118	81	35	32
C 179....	47	54	33	40
	300		150	

A preliminary test of the qualities of this purple stock was made by out-crossing a male to a wild female and carefully examining all F_2 flies (table 30). The F_2 showed only purple (150) and wild-type flies (300) as expected, but the ratio was 1 : 2 instead of 1 : 3. While this deviation was significant (4.1 times the probable error), it indicated a peculiarity of the wild parent rather than of the purple, and was not further regarded. The vestigial stock used was that from which purple itself was derived. It had been examined frequently and seemed to be clean.

The question of crossing-over in the male was the first point attacked. Complementary P_1 matings were made (June 13, 1913) by crossing purple vestigial to wild ("coupling") and by crossing purple by vesti-

gial ("repulsion"). F_1 males from these matings were back-crossed singly to purple vestigial females from the stock. The parents were in several cases transferred at the end of 10 days to fresh culture-bottles and second broods then raised.

TABLE 31.[1]—*B C. offspring given by the F_1 wild-type sons, from the out-cross of a purple vestigial male to a wild female, when back-crossed to purple vestigial females.*

July 7, 1913.	Non-cross-overs.		Cross-overs.	
	Purple vestigial.	Wild-type.	Purple.	Vestigial.
BQ.....	62	52	0	0
DR.....	113	141	0	0
DS.....	131	96	0	0
DS'.....	34	28	0	0
DT.....	89	68	0	0
DT'.....	33	22	0	0
DU.....	90	112	0	0
Total	552	519	0	0

[1]This table and table 32 were included by Morgan in his paper on "No crossing-over in the male of *Drosophila* . . ." Biol. Bull., April, 1914, pp. 200 and 201.

The offspring from the "coupling" experiment (5 pairs, both broods, table 31) gave a total of 1,071 flies, not one of which was a cross-over, and the "repulsion" experiment (3 pairs, both broods, table 32) added 704 more (total, 1,775), not one of which was a cross-over. Since these were back-cross experiments, there was no masking of results possible, and cross-over gametes had every opportunity to reveal

TABLE 32.—*B. C. offspring given by F_1 wild-type sons, from out-cross of purple male to vestigial female, when back-crossed to purple vestigial females.*

July 7, 1913.	Non-cross-overs.		Cross-overs.	
	Purple.	Vestigial.	Purple vestigial.	Wild-type.
DV.....	62	42	0	0
DV'.....	70	78	0	0
DW.....	61	53	0	0
DX.....	66	103	0	0
DX'.....	79	90	0	0
Total	346	358	0	0

themselves had any been formed, so that each fly recorded above is a true non-cross-over. While the total absence of cross-overs in these repetitions of the male test did not prove that the apparent cross-overs in the original test were not genuine, it added to the already large body of evidence which showed that they were at least aberrations from the normal condition.

The second point attacked was the amount of crossing-over in the female between the loci purple and vestigial. The same two complementary crosses that had furnished the material for the male tests just given were used as the source of the females to be tested. F_1 daughters from these two matings were back-crossed singly to purple-vestigial males, with the results given below.

BALANCED INVIABILITY—COMPLEMENTARY CROSSES.

The reason why both "coupling" and "repulsion" experiments were made is that by combining the two sets of data one can calculate a linkage value more nearly free from the errors due to disproportionate inviability of any class (Bridges, 1915, Muller,1916). Within each back-cross the inviability effects due to a given mutant form are largely neutralized. Since the inviable form occurs both as a cross-over and as a non-cross-over, both of these classes are lowered, but lowered proportionately, so that the linkage ratio remains practically

TABLE 33.—*B. C. offspring given by F_1 wild-type daughters, from out-cross of purple vestigial male to wild female, when back-crossed to purple vestigial males.*

July 5, 1913.	Non-cross-overs.		Cross-overs.		Per cent of cross-overs.	Change with age.
	Purple vestigial.	Wild-type.	Purple.	Vestigial.		
DA.....	178	202	16	16	7.8	.....
DA'.....	152	227	13	14	6.6	−1.2
DB.....	91	100	18	13	14.0	.....
DB'.....	69	104	12	8	10.3	−3.7
DC.....	165	150	17	19	10.3	.....
DC'.....	191	216	18	17	7.9	−2.4
DD.....	140	149	20	15	10.8	.....
DD'.....	116	122	9	4	5.2	−5.6
DE.....	191	214	20	19	9.0	.....
DE'.....	196	229	11	22	7.2	−1.8
DF.....	202	226	20	22	8.9	.....
DF'.....	197	228	25	20	9.6	+0.7
DG.....	105	158	17	17	11.4	.....
DG'.....	188	232	17	14	6.9	−4.5
DH.....	123	140	26	30	17.6	.....
DH'.....	129	179	11	20	9.1	−8.5
Firsts....	1,195	1,339	154	151	10.7	.....
Seconds..	1,238	1,539	116	119	7.8	−2.9

undisturbed. This internal balancing holds less well for combinations of characters; for any given combination occurs in an experiment either as a cross-over or as a non-cross-over, but not as both, and should any combination have an inviability disproportionate to that of the component mutant forms, then the cross-over value would be disturbed. The remedy for this condition is to balance the experiments in which a relatively inviable class occurs as a cross-over by an equal amount of data in which this same class is a non-cross-over. It

is often not convenient or possible to have complementary crosses of equal weight; but whatever is done in that direction, however little, is of advantage, and even a partially balanced result is to be preferred to one from only one type of cross. With improvements in culture methods, inviability effects have been very much reduced everywhere. Also, with the great increase in the number of mutations, there is now provided an abundance of forms which show only negligible inviability. Our regular work utilizes only these viable forms, and except for very special purposes those mutants which show more than a slight inviability are avoided.

TABLE 34.—*B. C. offspring given by F_1 wild-type daughters, from the out-cross of a purple male to a vestigial female, when back-crossed to purple vestigial males.*

July 5, 1913.	Non-cross-overs.		Cross-overs.		Per cent of cross-overs.	Change with age.
	Purple.	Vestigial.	Purple vestigial	Wild-type.		
DI.....	157	178	26	21	12.3	.....
DI'.....	200	165	12	14	6.7	−5.6
DJ.....	198	176	23	23	11.0	.....
DJ'.....	242	195	19	26	9.3	−1.7
DK.....	252	227	34	38	13.1	.....
DK'.....	198	178	26	20	10.9	−2.2
DM.....	205	158	27	32	14.0	.....
DM'.....	213	246	14	23	7.4	−6.6
DN.....	66	54	6	11	12.4	.....
DN'.....	66	64	4	7	7.8	−4.6
DO.....	189	172	30	32	14.6	.....
DO'.....	217	225	13	18	6.5	−8.1
Firsts....	1,067	965	146	157	13.0	.....
Seconds..	1,136	1,073	88	108	8.1	−4.9

The first back-crosses of purple-vestigial had shown a marked inviability for vestigial and a slight amount for purple. The new back-crosses showed practically no inviability for purple and a very moderate amount for vestigial, but still enough to repay the added labor required by the balancing cross. As in the first back-cross test of the female, the linkage shown was fairly strong. Since the linkage shown by second broods proved to be different from that of firsts, only first broods will be considered for the moment. The "coupling" experiment (table 33) gave a total of 2,839 first-brood flies, of which 305 or 10.7 per cent were cross-overs. The "repulsion" first broods (table 34) gave a total of 2,335 flies, of which 303 or 13.0 per cent were cross-overs. When the first brood data from both these experiments are combined, so that the inviability is balanced, the cross-over value is 11.8 (608 cross-overs in a total of 5,174.)

These two component cross-over values differed slightly from each other and from the value (9.1) obtained in the original experiment. It may be questioned whether the difference in the cross-over values

was entirely due to inviability. Slight differences of this order, but many of them undoubtedly significant, are continually appearing in our work. Other known causes of linkage variation besides inviability are: differences in the age of parents (Bridges, 1915) or of the temperatures at which the experiments are conducted (Plough, 1917), or mutant "cross-over" genes (Sturtevant, Muller, and Bridges), and probably to several other internal and external factors not yet analyzed. The best that can be done in correction is to calculate mean values from as many experiments as possible where none of the recognized causes of variation are especially active, and thus obtain a sort of composite picture of the "normal" condition.

THE VARIATION IN CROSSING-OVER WITH AGE.

The reason for raising second broods in these experiments was to obtain more offspring from each female and thus secure a more trustworthy index of the genetic behavior of individual. This practice was extended to all the work at this time, and was continued until a comparison of the cross-over values of the first and second broods brought out a remarkable relation in the cases involving the second chromosome. There was found to be a change in the amount of crossing-over, so that both in the totals for each experiment and in a great majority of the individual cultures the cross-over value had fallen significantly. Equally surprising was the fact that there was no such change in the case of the first chromosome, and this added another proof of the distinctness of our linkage groups—that is, of the individuality of the chromosome involved. The first case in which this decrease for the second chromosome was clearly seen was that of the back-cross tests of the purple vestigial linkage given in tables 33 and 34. Of the 8 females whose tests are given in table 33, seven showed a decrease in percentage of crossing-over and only one (F) showed an increase, which, however, was smaller in amount than the smallest of the decreases. In the complementary case "repulsion" (table 34) all 6 females showed a decided drop. The totals likewise reflected this same change; the decreases were 2.9 and 4.9 units respectively. The cross-over value calculated from the balanced second broods was 8.0, a decrease of 3.8 units, or, compared with the corresponding cross-over value (11.8) from the balanced first broods, a 32 per cent decrease from the normal amount. Many other experiments have confirmed the fact of change in crossing-over frequency with the age of the mother, and a partial analysis has been made.

THE LOCUS OF PURPLE—A TWO-POINT MAP.

The repetition of the purple vestigial back-crosses was not carried out until the summer of 1913; meanwhile considerable progress had been made with the mapping of the second chromosome. The test

of the amount of crossing-over in the female between the loci purple and vestigial (table 29) had given a cross-over value of 9.1 units. The next cross-over value to be worked out was that of black vestigial as about 20 units (Morgan, 1912). With these two values alone it was not possible to determine the relative order within the chromosome of the three loci involved; it was apparent that black was farther away from vestigial than from purple, but it could not be told whether it lay

TABLE 35.—*P_1 mating, purple ♂ × black ♀; F_1 mating, wild-type ♀♀ and ♂♂.*

Oct. 24, 1912. F_2,	Wild-type.	Black.	Purple.	Black purple.
C 68.....	248	137	136	0
C 69.....	278	103	157	0
C 70.....	158	60	78	0
Total	684	300	371	0

on the same or on the other side of vestigial from purple. The black purple value should be one of two values depending on the order of the genes; it should be an approximation to either the sum (20+9=29) or the difference (20−9=11) between the black vestigial and the purple vestigial values. To carry out a back-cross experiment for black and purple it was first necessary to make up the double recessive. No easy task was anticipated in this, for it had just become known that on account of no crossing-over in the male no double recessive could be

TABLE 36.—*P_1 mating, purple ♂ × black ♀; B.C., F_1 ♀ × black purple ♂.*

B. C. of ♀, Dec. 12, 1912.	Non-cross-overs.		Cross-overs.	
	Black.	Purple.	Black purple.	Wild-type.
C 174....	320	339	13	18
II 1.....	33	43	3	4
Total..	353	382	16	22

obtained in F_2, as in fact none was (table 35). As expected, the F_2 ratio approximated 2 : 1 : 1 : 0. Three sorts of F_2 mass-culture matings were made: black × black, purple × purple, and black × purple. Of these matings the last type is by far the most valuable, since in case one of the flies happened to come from a black purple cross-over egg × a black sperm it would give some purple offspring when crossed to purple; and these, inbred, would give the required black purples as a quarter of the next generation. Likewise, if one of the purples had come from a cross-over black purple egg, the black × purple cross would produce some blacks that would give the required

black purples upon inbreeding. If both the black and the purple chosen happened to have come from cross-over eggs, then the double would be produced in F_3 directly. In case none of the parents proved to be from cross-over gametes, than at least the F_3 wild-type flies are equivalent to the F_1 and would save a generation in the repetition. The other two types of crosses would give a favorable result only if both parents happened to be from cross-over eggs, in which case the double would appear among their progeny.

TABLE 37.—*P_1 mating, purple, ♂ × black ♀; B. C., F_1 ♂ × black purple ♀.*

B.C. of ♂, Dec. 12, 1914.	Non-cross-overs.		Cross-overs (♂ test).	
	Black.	Purple.	Black purple.	Wild-type.
II 2.....	74	71	0	0

It so happened that one of the black × black crosses gave a few black purples in F_3 directly and from these a stock was made for use in back-crossing. At the same time a P_1 mating of a black male to a purple female was started to furnish the required F_1 heterozygotes. A single test of the F_1 male showed, as expected, no crossing-over whatever (table 37).

Two back-cross tests of the female gave a total of 773 flies, of which 38 or 4.9 per cent were cross-overs (table 36). Of the two expected values, that of 30 is excluded entirely, and that of 10 is approximated, though not very closely. On this basis, the order of these genes is black, purple, vestigial, and not black, vestigial, purple.

A THREE-POINT BACK-CROSS, BLACK PURPLE CURVED, WITH BALANCED INVIABILITY.

Most of the linkage experiments up to this time had involved only two loci, as the three just cited, namely, purple vestigial, black vestigial, and black purple. It was now realized that a more complex type of experiment involving all three loci at once would yield returns whose value far outweighed the greater labor entailed. Thus, a multiple back-cross for black purple vestigial would give linkage data upon all three cross-over values simultaneously, and these values would be strictly comparable, since there would be no possibility of discrepancies due to different conditions of culture or parentage. Accordingly, the simple black purple back-cross was done on a scale only large enough to decide between two possible values and thus show what was the order of the three loci. A knowledge of this order is of great advantage in synthesizing the multiple recessive. It was found, as already stated, that black and vestigial are the two farthest apart and

the mating was accordingly arranged so that a cross-over anywhere within this whole distance would give the required triple form. That is, black purple and purple vestigial were mated together and the resulting purple offspring $\left(\frac{b\ p_r\ +}{+\ p_r\ v_g}\right)$ were inbred. The F_2 black

TABLE 38.—*P_1 mating, black purple vestigial ♂ × wild female; B. C. mating F_1 wild-type ♀ × black purple vestigial ♂.*

Jan. 9, 1914.	$\frac{b\ p_r\ v_g}{}$		$\frac{b\ \vert}{\vert\ p_r\ v_g}$		$\frac{b\ p_r\ \vert}{\vert\ v_g}$		$\frac{b\ \vert\ v_g}{\vert\ p_r\ \vert}$	
	Black purple vestigial.	Wild-type.	Black.	Purple vestigial.	Black purple vestigial.	Vestigial.	Black vestigial.	Purple.
II 141.....	89	140	3	1	11	12	1	1
II 142.....	118	137	3	3	15	9	.....	.....
II 143.....	61	78	2	4	12	11	.....	.....
Total....	268	355	8	8	38	32	1	1

TABLE 39.—*P_1, black × purple vestigial; B. C. test of F_1 ♀ ♀ singly.*

Mar. 9, 1914.	$\frac{b}{p_r}$	$\frac{}{v_g}$	$\frac{b\ \vert\ p_r\ v_g}{\vert}$		$\frac{b\ \vert\ v_g}{p_r\ \vert}$		$\frac{b\ \vert\ p_r\ \vert}{\vert\ \vert\ v_g}$	
88.....	114	96	10	10	7	15	1	1
103.....	92	81	15	5	12	16	1	0
104.....	99	98	11	15	8	17	0	0
115.....	97	66	2	12	14	13	0	1
116.....	164	77	6	15	12	19	2	0
Total....	566	418	44	57	53	80	4	2

TABLE 40.—*P_1, black vestigial × purple; B. C. test F_1 ♀ ♀ singly.*

Mar. 10, 1914.	$\frac{b\ \vert\ v_g}{p_r\ \vert}$		$\frac{b\ \vert\ p_r}{\vert\ v_g}$		$\frac{b\ \vert}{p_r\ \vert\ v_g}$		$\frac{b\ \vert\ p_r\ \vert\ v_g}{\vert\ \vert}$	
101.....	85	126	10	7	23	15	1	0
102.....	137	133	13	13	16	23	0	0
112.....	67	68	7	7	12	8	0	1
113.....	92	137	13	8	21	9	1	1
Total...	381	464	43	35	72	55	2	2

purples and purple vestigials were crossed together in several mass-cultures, and in F_3 some triples occurred, showing that some of both kinds of F_2 flies used had come from cross-over eggs. A better method would have been to back-cross the F_1 female by a black-vestigial male. In this case every black vestigial cross-over would be known to be of the composition $\left(\frac{b\ p_r\ v_g}{b\ +\ v_g}\right)$, and these inbred would give the pure

triple without the chance of failure entailed by method actually used. A stock of black purple vestigial was made from the triple recessives that hatched in F_2 (March 1913).

In carrying out the triple back-cross, the principle of balancing the inviability by complementary crosses was applied. To completely balance a three-locus experiment requires four types of crosses, so that every class may appear in each of the four cross-over categories, namely, (0) non-cross-overs, (1) cross-overs in the first region, that

TABLE 41.—*P_1, black purple × vestigial; B. C. of F_1 ♀ ♀ singly.*

Oct. 28, 1914.	b p_r / v_g		b / p_r v_g		b p_r / v_g		b / p_r / v_g	
654.....	130	152	10	5	14	10	0	0
670.....	124	111	10	8	11	14	1	1
671.....	137	138	15	6	12	30	2	2
672.....	131	154	7	10	18	12	0	1
673.....	162	151	11	7	12	13	2	0
674.....	159	162	8	7	16	24	0	2
Total....	843	868	61	43	83	103	5	6

TABLE 42.—*Summary of the four types of black purple vestigial back-cross, with inviability balanced.*

Combinations.	0		1		2		1, 2		Total.
b p_r v_g	268	355	8	8	38	32	1	1	711
	623		16		70		2		
b									
p_r v_g	566	418	44	57	33	80	4	2	1,224
	984		101		133		6		
b p_r									
v_g	843	868	61	43	83	103	5	6	2,012
	1,711		104		186		11		
b v_g	381	464	43	35	72	55	2	2	1,054
p_r	845		78		127		4		
Total.....	4,163		299		516		23		5,001

between black and purple, (2) cross-overs in the second region, that between purple and vestigial, and (1, 2) double cross-overs, the simultaneous occurrences of crossing-over in both regions. Thus, the cross of black purple vestigial by wild and the back-cross of the F_1 wild-type daughters by the triple-recessive male gave one of the four types of crosses (table 38). The other types of experiment carried out were black by purple vestigial (table 39), black vestigial by purple (table 40), and black purple by vestigial (table 41). In order that these four crosses should balance closely, the same number of cultures (6)

was started in each case. A few of these cultures failed, and the total data in the separate experiments are consequently not in equal amounts. The balance is for this reason not perfect, though such partially balanced results are far better than an equal amount of data secured from only one of the four possible types of experiment. Additional cultures could have been raised until a balance was reached, and such a practice has been followed in other cases, for example, the vermilion sable forked case reported by Morgan and Bridges (1916). A summary of these complementary crosses appears in table 42, from which the following balanced cross-over values are calculated: black purple 6.4, purple vestigial 10.8, and black vestigial 16.3.

COINCIDENCE.

Another and very important advantage of these more complex crosses is that the process of double crossing-over can be examined. Thus there were 23 double cross-overs, or 0.46 per cent of the total flies. If the proportion of double cross-overs were determined by chance alone the percentage should have been 6.4 per cent of 10.8 per cent or 0.69 per cent of the total. The observed per cent of coincident cross-overs (0.46) is only 61 per cent of the theoretical per cent (0.69). This percentage, 61, is called the "coincidence" for black purple vestigial. This index can be more conveniently calculated directly from the back-cross numbers as follows (see Weinstein, 1918):

$$\frac{\text{No. of doubles} \times \text{Total flies} \times 100}{\text{Total firsts} \times \text{Total seconds}} = \frac{23 \times 5001 \times 100}{322 \times 539} = 61.3$$

THE RELATION BETWEEN COINCIDENCE AND MAP-DISTANCE.

The coincidence of 61 observed in this case is relatively very high. A coincidence under 5 is expected for cases in the first chromosome where similar map-distances are involved. This higher coincidence may mean that for some reason the freedom of crossing-over is much less in this region of the second chromosome than it is in the first chromosome. The 17.5 units of map-distance between black and vestigial may correspond to as great a length of actual chromosome as is involved in cases in the first chromosome where the coincidence is the same, but the map-distances are nearly three times as great. On the other hand, instead of the higher coincidence being due to a lower "coefficient of crossing-over," it may be due to a relatively short "average internode." The length of chromosome represented by a given map-distance may be the same in the two regions compared, but in the second chromosome the mechanism of double crossing-over may not require so long a section of chromosome between successive cross-overs. If the average length of the internode was shorter because of this closer spacing of doubles, then a greater proportion of doubles would occur in the given region from black to vestigial, and coincidence would be correspondingly higher. However, the interest

of these problems in double crossing-over is out of all proportion to our progress in their solutions.

It seems likely that a more satisfactory method of expressing these relations may be derived than is provided by the present formula; the new formulation must take account of separate factors analyzable in the process and permit their adequate representation. The conclusions based on the old formula must be regarded as provisional.

THE USE OF PURPLE IN MAPPING OTHER GENES—CURVED, STREAK, ETC.

The "map" of the second chromosome began to be useful when the order and spacing for the three genes, black, purple, and vestigial, were roughly established by the determination of the third value, that for black purple (December 1912). The three-locus experiment just given provided more accurate measures of the map-distances involved. The preparation of the multiple recessive and of the P_1 stocks delayed the completion of this experiment for nearly a year (January 1914). Meanwhile, these provisional locations were used as the basis for locating other genes more closely. The first of these was curved. Bridges and Sturtevant (Biol. Bull., 1914) soon found (January 1913) that black and curved gave approximately 23 per cent of crossing-over. The next point to be determined was the relation of curved and one of the other two mutations whose loci had been mapped. Both of these tests were used, since each offered advantages; the chief disadvantage was that vestigial interferes with the classification of curved, so that it is impossible to distinguish between the simple vestigials and the vestigial curved class.

The purple curved test was undertaken by Bridges, who prepared to run a three-point back-cross involving black, purple, and curved. The first step was the synthesis of the purple curved double recessive. As soon as this was obtained it was turned over to Mr. W. S. Adkins, who ran a preliminary back-cross test of the simple purple curved crossing-over, and found that there was about 18 per cent of crossing-over. This enabled us to determine the relation of curved to the other three genes. The purple curved value of 18 showed that curved was closer to purple than to black (black curved = 23) and that purple and vestigial were therefore "to the right" of black. Curved was further to the right than vestigial, since black and vestigial gave only about 18 per cent of crossing-over.

The vestigial curved distance was tested by Sturtevant, who found that there was about 8.5 per cent of crossing-over. Because of the difficulty of classification already referred to, it was not thought worth while to run these tests on a large scale. However, vestigial is itself accurately mapped and is nearer to curved than purple is, and these considerations are strong enough so that the vestigial curved tests will

ultimately be extended and may furnish the main basis for the accurate mapping of curved.

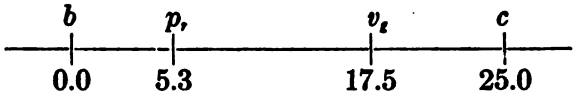
Meanwhile, the purple curved test, while less satisfactory because of the longer interval with the attendant correction necessary for double crossing-over, was more readily handled, and this led to a rapid accumulation of data on the purple curved cross-over value. The black purple curved triple recessive was obtained (May 1913), and the back-cross itself carried out. Thirteen of the F_1 wild-type daughters from the cross of black purple curved to wild were tested by back-crossing singly to males of the triple form. These same parents were at the end of 10 days transferred to fresh culture-bottles and second broods were raised. The details of the data of these cultures have already been published (Bridges, 1915), and we need repeat here only the totals for the first broods (table 43).

TABLE 43.—*Total offspring in the first broods of the black purple curved × wild back-cross (details published J. E. Z., July 1913, p. 8).*

	b p_r c		b / p_r c		b p_r / c		b / c p_r		Total.	b p_r value.	p_r c value.	b c value.
Aug. 24, 1913...	1,476	1,577	96	74	339	330	19	23	3,934	5.4	18.1	21.3

The second broods confirmed on a large scale the fact first brought to light in the purple vestigial back-cross (table 33), that in the second chromosome the amount of crossing-over changes with the age of the mother (see Bridges, 1915).

The numerical relations in the first broods of this experiment confirmed the position of curved as already mapped. A map of the second chromosome was constructed on the basis of the data then on hand (October 1913), and was as follows:



The black purple curved back-cross was carried out in only one of the four possible ways, and is therefore unbalanced. However, the results showed that inviability was negligible. What little deviation there was from expectation can be attributed to curved, which still appeared to the extent of 97 flies for every 100 expected.

ALTERNATED BACK-CROSSES.

In cases where only one type of back-cross is to be made, the poorest type is that in which all the mutants are together, as was the case in the black purple curved × wild experiment just cited. The flies having the most mutant characters are relatively the least viable, and this type of cross furnishes the highest proportion of such individuals.

The best type is that known as "alternated," where the successive genes alternate between the two chromosomes $\frac{b + c}{+ p_r +}$, so that the maximum of evenness of distribution of characters is attained. It requires double crossing-over to put all the mutant characters in the same individual, and accordingly the alternated experiment gives a minimum number of the combinations that are most inviable. This principle becomes still more important in more complex experiments, as, for example, $\frac{S' + b + c + s_z}{+ d + p_r + p_z +}$.

The next mutant whose locus was mapped with reference to purple as a base was "streak," a dominant character which shows as a dark streak from the scutellum forward along the dorsal region of the thorax.

TABLE 44.—*Streak purple curved back-cross data.*

Combinations.	Total.	0	1	2	1, 2	Coincidence.
$\frac{S_k}{p_r \quad c}$	878	435	247	127	69	98.0
$\frac{S_k \quad p_r}{c}$	929	496	254	117	62	102.0
Total.....	1,807	931	501	244	131	100.2

The triple black-cross streak purple curved, which was made in two of the four possible ways and is therefore partially balanced (table 44), showed that streak is far to the left of purple, that is, beyond black, and in the opposite direction from vestigial and curved. The streak purple cross-over value was 35.0, which showed that streak is so far to the left of purple that only an approximate calculation of its position could be made from the data. In a region of such length the correction to be supplied because of double crossing-over is quite large and correspondingly inexact. On the basis of data that have since become available it appears that there is about 37.3 per cent of separation between streak and purple. Purple has since played an important rôle in the mapping of several other genes, the details of which will appear in accounts of these mutations.

A SUMMARY OF THE LINKAGE DATA INVOLVING PURPLE.

Besides the data reported in the various sections of this paper, there are available data from three other principal papers: Bridges's study of age variation in crossing-over (J. E. Z., 1915), Muller's study of crossing-over by means of the progeny test (Am. Nat., 1916), and Plough's study of temperature variations in crossing-over (J. E. Z., 1917).

Table 45 gives a detailed summary of all these data collected according to two loci calculations. The black purple cross-over value of 6.2 based on 48,931 flies places the locus of purple at 6.2 units to the right of black, or at 52.7 when referred to star as the zero-point.

TABLE 45.—*Summary of purple cross-over data.*

Loci.	Total.	Cross-overs.	Per cent.	Date.	By —	Reference.
Star purple....	6,766	3,010	44.5	July 11, 1915	Bridges....	$S'; \frac{S'}{p_r\ c\ s_p}$ B.C., 1sts; 1836–1894.
	1,027	413	40.2	Aug. 24, 1916	Do.	$S'; \frac{S'\ \ p_r}{S_k\ \ d}$ B.C.; 4999–5110.
	362	138	38.1	July 21, 1917	Do.	$S'; \frac{S'}{p_r}$ B.C.; 7391–2.
	8,155	3,561	43.7			
Streak purple..	1,807	632	35.0	Nov. 6, 1913	Bridges....	*Sk*; *Sk pr c* balanced B.C.; II 103–124.
	462	137	29.7	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	396	114	28.8	Aug. 24, 1916	Bridges....	$S'; \frac{S'\ \ p_r}{S_k}$ B.C., *Sk* only 4999–5110.
	2,665	883	33.1			
Dachs purple..	462	97	21.0	May —, 1914	Muller....	Am. Nat., 1916, p. 422.
	1,027	196	19.1	Aug. 14, 1916	Bridges....	$S'; \frac{S'\ \ p_r}{S_k\ \ d}$ B.C.; 4999–5110.
	1,489	293	19.7			
Black purple...	773	38	4.9	Dec. 12, 1912	Bridges....	p_r; *b* p_r B.C.; C 174–II 2.
	3,934	212	5.4	Aug. 28, 1913	Do.	p_r; *b* p_r *c* B.C., 1sts; II 58–II 88.
	5,001	322	6.4	Jan. 9, 1914	Do.	p_r; *b* $p_r\ v_g$ balanced B.C.; II 141–674.
	462	26	5.6	May —, 1914	Muller.....	Am. Nat. 1916, p. 422.
	36,622	2,214	6.0	Jan. 5, 1915	Plough....	J.E.Z. 1917, total *b* p_r *c* B.C. control.
	2,139	214	10.0	Mar. 5, 1917	Do.	J.E.Z. 1917, total *b* $p_r\ v_g$ B.C. control.
	48,931	3,026	6.2			
Purple vestigial	825	79	9.1	July 16, 1912	Bridges....	p_r; $p_r\ v_g$ B.C.; B 36.1–B 39.2.
	2,839	305	10.7	July 5, 1913	Do.	p_r; $p_r\ v_g$ B.C.; DA–DH.
	2,335	303	13.0	July 5, 1913	Do.	$p_r; \frac{p_r}{v_g}$ B.C.; DI–DO.
	5,001	539	10.8	Jan. 9, 1914	Do.	p_r; *b* $p_r\ v_g$ balanced B.C.; II 141–674.
	462	60	13.0	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	2,139	323	15.1	Mar. 5, 1917	Plough....	J.E.Z., 1917, total *b* $p_r\ v_g$ B.C. controls.
	13,601	1,609	11.8			
Purple curved.	3,934	711	18.1	Aug. 24, 1913	Bridges....	p_r; *b* p_r *c* B.C. 1sts; II 58–II 88.
	1,807	375	20.7	Nov. 6, 1913	Do.	S_k; $S_k\ p_r\ c$ balanced B.C.; II 103–II 124.
	462	90	19.5	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	952	182	19.1	Aug. 24, 1914	Bridges....	*sp*; $p_r\ c\ s_p$ B.C.; 452–508.
	36,622	7,222	19.7	Jan. 5, 1915	Plough....	J.E.Z., 1917, *b* p_r *c* B.C. controls.
	6,766	1,508	22.3	Feb. 11, 1915	Bridges....	$S'; \frac{S'}{p_r\ c\ s_p}$ B.C. 1sts; 1836–'94.
	593	117	19.7	Feb. 8, 1916	Do.	*lIIa*; $p_r\ c\ s_p$ F_2; 3203–'8.
	51,136	10,205	19.9			
Purple plexus..	344	164	47.7	Feb. 29, 1916	Bridges....	*lIIa*; $p_r\ p_z\ s_p$ F_2; 3535–'53.
Purple arc.....	2,625	1,066	40.6	Oct. 24, 1914	Do.	$a; \frac{p_r}{a\ s_p}$ B.C. 637–686.

TABLE 45.—*Summary of purple cross-over data*—continued.

Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Purple speck..	462	218	47.2	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.
	952	410	43.1	Aug. 24, 1914	Bridges....	s_p; p_r c s_p B.C.; 452–508.
	2,625	1,166	44.4	Oct. 24, 1914	Do.	$a; \frac{p_r}{a \quad s_p}$ B.C.; 637–686.
	6,766	3,130	46.3	July 11, 1915	Do.	$S'; \frac{S'}{p_r \quad c \quad s_p}$ B.C. 1sts; p. 1836–'94.
	259	95	36.7	Feb. 7, 1916	Do.	$l_{IIa}. \frac{p_r \quad s_p}{l_{IIa}}$ F_2; 3168–.....
	565	279	49.4	Feb. 8, 1916	Do.	l_{IIa}; b c s_p F_2; 3203–'8.
	356	176	49.4	Feb. 29, 1916	Do.	l_{IIa}; p_r p_x s_p F_2; 3535–'53.
	11,985	5,474	45.7			
Purple balloon.	462	218	47.2	May —, 1914	Muller.....	Am. Nat., 1916, p. 422.

SPECIAL PROBLEMS INVOLVING PURPLE—AGE-VARIATIONS, COINCIDENCE, TEMPERATURE-VARIATIONS, CROSS-OVER MUTATIONS, PROGENY TEST FOR CROSSING-OVER.

We have already seen how the study of the age-variation in crossing-over for the second chromosome began with the purple vestigial back-cross (p. 181) and was continued and confirmed by the black purple curved triple back-cross (p. 185). Some of the early data suggested that the drop in the second broods was followed by a recovery and perhaps even by a rise in later broods (Bridges, 1915).

To gain further light on the course of the variation throughout the life of the fly a special and extensive experiment was continued through four broods. The entire length of the chromosome was covered by the loci chosen $\left(\frac{S'}{p_r \; c \; s_p}\right)$.

This experiment showed the normal cross-over values for the first broods, the usual drop for the second broods, and a slight continued drop for the third and fourth broods. However, the experiment proved inconclusive because of two ill-adaptations: the chromosome distances involved were so long (*e. g.*, S' $pr = 52.7$) that real changes could be concealed by a concomitant change in double crossing-over, and further because the 10-day broods gave only four points on the curve of age-variation, each point representing only the net change for a 10-day period, while the real underlying curve may have changed its course so that an unknown part of each 10-day period may have been a fall and the rest a rise.

The original black purple curved experiment had avoided one of these difficulties in that the black-purple distance is so short that there

is probably no double crossing-over whatever within it. The second difficulty was met by Plough in his similar studies on the temperature variation of linkage, by transferring his parents every 2 days instead of every 10 (Plough, 1917). As one of his control experiments Plough ran a black purple curved back-cross of 13 pairs, transferring each pair to a fresh culture-tube every 2 days as long as the female lived (table 14, Plough, 1917). The plotted curve of the percentages of crossing-over between black and purple shows an initial high value (8 per cent) which during the first 9 days falls rapidly at first and then more slowly to a low value (5 per cent), which is maintained with little change to about the sixteenth day. A sharp rise then sets in which reaches its maximum (8 per cent) at about the twenty-first day. The succeeding fall is again slow, reaching its minimum (3.5 per cent) at about the thirtieth day. Beyond this point the curve again rises slightly; but the data were too few to be significant beyond about the twenty-fifth day. While there was some variation in the amount and rapidity of these changes in the various individual curves, all showed the same typical rhythm, which must be the expression of fundamental physiological changes in the development of the female. It seems possible and probable that these successive falls and rises are not effects of a single continuously varying physiological process, but are rather to be explained as separate phenomena caused by the lapse of certain conditions and the subsequent onset of new causes. These changes may therefore be really discontinuous and the rhythmic curve only a succession of independent but overlapping variations.

The most interesting feature of the age-variation is the bearing it has on the problem of double crossing-over and the underlying problems of the nature of crossing-over. The more that consideration has been given to this problem of double crossing-over in relation to chromosome and to map-distances, the more involved it has appeared, so that no evidence upon these points can be neglected. The special value of such cases as that of age-variation is that they enable one to compare two different conditions but with the elimination of one important variable; for the actual chromosome-distance between such genes as black and purple is maintained constant, so that any variations that occur in map-distance, coincidence, etc., must be due to variations in one or more of the other factors. This relation was discussed in connection with the original two-brood black purple curved cross (Bridges, 1915), and it was pointed out that the rise in coincidence concomitant with the fall in crossing over meant that the internode length had changed—that the two cross-overs of a double no longer included the same average length of chromosome, but a longer length. The coincidences of the $\frac{S'}{p_r\ c\ s_p}$ case support this view, but in neither

case are the data conclusive. A calculation of the coincidence shown by Plough's black purple curved 2-day tube-cultures has provided data considerably more satisfactory, though still subject to a high probable error. On the basis of all the data it is probable, not only that coincidence varies with age, but that the curve of age-variation in coincidence is roughly the mirror image of the curve of age variations in crossing-over. While it seems probable that at least part of the explanation of the age-variations both in crossing-over and in coincidence has been found in an internode variation as suggested, yet in any case there is provided evidence of a common cause that should repay further analysis.

A second problem involving purple and very closely allied to the age-variation in conception, material, methods, and bearing is that of the temperature-variation described by Plough (1917). Since the genic constitution of a female showing the age-variation is constant throughout the course of this variation, the immediate causes of the variations must be regarded as environmental differences arising through rhythmic changes in the physiological processes of nutrition and development. While the crossing-over variations due to age and to specific genes were effected through environmental changes arising internally, they suggested the possibility that similar variations might be initiated by environmental changes arising externally. Plough found that exposure to abnormally high or low temperature actually did produce linkage change even more extreme than those due to age changes. Black-purple-curved back-cross cultures were paired at various temperatures from 9° to 32° C. When the black-purple cross-over values were plotted it was seen that at a low temperature (9°), crossing-over is very free (14 per cent) and becomes even more free at 13° (18 per cent). The amount of crossing-over then falls away very rapidly, and at 18° is nearly the normal value (6.0 per cent). This value is maintained to about 27°, or throughout the range of "room" temperature at which the breeding work is ordinarily conducted. At 29° the crossing-over is slightly freer than normal, but between 29° and 31° the amount of crossing-over nearly trebles (18.2 per cent). This extraordinarily sharp and extensive rise is followed by a slight fall at 32° (15.4 per cent). Above this temperature and below 9° C. it was found that the flies either died or produced too few offspring to be workable. It seems probable that here also these two sharply marked maxima, separated by a long interval of no or slight change, may represent two distinct phenomena.

When the coincidences are calculated for these various temperatures it is seen that the curve of temperature-variation of coincidence is a slightly rising but practically straight line cutting alike through both of the maxima and the normal interval. This is a significant difference

from the relation previously observed in the age-variations, and would seem to indicate that the age and temperature variations were accomplished by different mechanisms—by effects upon different physiological factors. Those double cross-overs that do occur have the same distribution along the chromosome at all temperatures, which shows that the method of handling the chromosomes is unchanged. In accordance with the analysis already given (p. 188), the cause of the temperature variations in crossing-over is to be sought rather in variation in the coefficient of crossing-over—in the crossing-over capacity of the chromosome itself, because of some variations in its structure or framework. Just as in the relation between the coincidence and temperature curves discussed before, the coincidence curve calculated for such a tube-count temperature-time curve apparently cuts through the rise and fall due to temperature instead of being altered concomitantly with it.

The conclusion just drawn from the failure of the temperature-variation to affect the coincidence, namely, that the change in the amount of crossing-over is probably due to a change in the physical properties of the chromosome substance, has an important bearing on the question of the stage at which crossing-over itself occurs, as follows: From a study of the time taken for the effects of exposure to abnormal temperature to become manifest or to disappear, Plough concluded that the effect was produced at one stage only in the development of the ovary, and that eggs which have not arrived at or have passed this critical stage are incapable of registering any temperature-variation. It was next argued that this critical stage is that at which crossing-over itself normally occurs. It seems certain that crossing-over does not take place before this critical stage is reached, but it does not follow that it might not occur at some stage between this and the maturation divisions, that is, at any later stage during the growth period. At the critical stage one of the factors which modifies the frequency of the crossing-over becomes fixed, but the crossing-over itself may occur later. As a crude analogy, the process of crossing-over might be likened to a machine, say a saw-mill. The rate at which boards are sawn depends, other factors remaining constant, upon the toughness of the log fed against the saws, which toughness is a physical property of the log fixed long previously.

The coincidence analysis indicates that the setting of the crossing-over machine has not been altered, but that the chromosome at a specific sensitive stage in its fabrication has been modified in one of its properties—its toughness, let us say—so that when it ultimately undergoes crossing-over the output is different.

It is quite possible that the crossing-over follows immediately after the determination of this property; indeed, from other lines of evidence

it seems probable that crossing-over occurs at a thin-thread stage, or at least that the characteristic transjunction is accomplished at a leptotene stage, such as occurs only in the early growth-period. But such a conception does not exclude the possibility that the crossing-over occurs at a four-strand stage, as is indicated by still other lines of evidence. To call such an early-growth-stage, thin-thread, four-strand hypothesis of crossing-over "chiasmatype" would be misleading, since the term is usually understood as applying to a late-growth-stage, thick-thread, four-strand condition. The term "tetraleptotenic" might be used for this type of crossing-over to distinguish it from both the dileptolenic and the chiasmatype hypotheses.

Plough's evidence that the critical stage and crossing-over occur after most or probably all of the oögonial divisions have been completed effectually disproves the reduplication hypothesis of crossing-over as far as any application to *Drosophila* is concerned, for the number of divisions required by that hypothesis is not available.

Purple has been extensively used in two other important studies on crossing-over—that of Sturtevant upon inherited crossing-over variations (Part III of this volume), and that of Muller in his progeny test of a multiple heterozygote in studying crossing-over. (Muller, 1916).

SUMMARY AND VALUATION OF PURPLE.

Of the 200 or more mutations in *Drosophila*, certain ones have proved especially useful as working tools because of excellent characteristics or favorable location in the chromosome. Certain others have an even higher interest because of their intimate connection with the development of principles or subjects that have now come to be the groundwork of every *Drosophila* experiment.

As a working tool the second-chromosome recessive eye-color purple has deserved its very extensive usage. In viability, fertility, productivity, and in the details of habit—ease of handling, activity, time of hatching, length of life, etc.—purple measures well up to the standard of the wild fly. In separability from the wild-type, purple is satisfactory both in certainty and in speed. The only failing in certainty is that arising from the occurrence in the same culture of a similar eye-color—a "mimic" or "pseudo-purple"—either by mutation or by introduction. However, the presence of a "mimic" is generally easily recognized and such difficulties in classification are only temporary. The ease and rapidity of separation fail to be satisfactory with purples older than about 3 days, though rarely is there any need for separations so delayed. The usefulness of purple has not been restricted by "masking" effects. Until very recently there has been no other readily workable second-chromosome eye-color so similar to purple in appearance as to prevent the use of both in the same experiment

without confusion between them. Purple is not so dilute that it would interfere with the classification of other eye-colors, as does "white" in the X chromosome; nor conversely is there any other second-chromosome eye-color so dilute, or morphological change so extreme, as to interfere with the classification of purple in flies possessing both characters. The recessiveness of purple seems to be complete and constant, so that the chance of confusion between it and the heterozygote is *nil*.

The locus of purple on the basis of very extensive data is 6.2 units to the right of black, or, referred to star as a base, at 52.7. Purple is not far from the middle of the second chromosome as mapped, and is thus within striking distance of mutant loci near either end or anywhere throughout the chromosome. Its closeness to black (which is the primary base in the mapping of the second chromosome, and another of the very best characters) furnishes a working distance which is short enough to exclude double crossing-over and long enough to avoid the excessive probable errors incident to very small percentages of cross-overs. Outside this black-purple section the second chromosome is as yet mostly mapped in distances too great or too small to handle satisfactorily in special tests. Furthermore, it appears that this purple region is peculiarly sensitive, as is proved by its exceptionally high double crossing-over (this paper), by its greater disturbance by age (Bridges, 1915; Plough, 1917), by temperature (Plough, 1917), and by its unique reaction to genetic variations in crossing-over (Sturtevant, Part III of this volume). The explanation of this sensitiveness is probably that this region is actually near the middle of the chromosome with the spindle fiber attachment, and that this middle region is the last part to undergo synapsis.

The number of subjects in the genetics of *Drosophila* toward whose early and continued development purple has contributed is surprisingly large.

In the field of mutation it gave with vermilion the first case in which "intensification" or "disproportionate modification" was recognized and made use of. It was the first of the class of "dark" eye-color mutations. It has been one of the most popular models in *Drosophila* for "mimic" mutations. The most striking "epidemic of mutation" or "mutating period" was that inaugurated by purple.

In the early experiments involving purple several other mutations arose, probably the most interesting of which was "extra bristles," which led to the study made by MacDowell on the effect of selection on bristle number.

In the attack upon the problem of "inviability" purple entered into the first experiment planned to include the balancing of inviability by complementary crosses. This practice was extended to involve three

locus experiments in the balancing of the black purple vestigial back-cross. The inviability of vestigial met with in the early purple vestigial crosses seems not to have been due primarily to "prematuration," "repugnance," or autosomal lethals, but probably to culture conditions, as shown by Carver (unpublished).

In the development of autosomal linkage, purple was involved in the first coupling F_2 back-cross test of crossing-over in the male, and likewise in the female. One of these back-cross tests of the male gave a few apparent cross-overs which prevented a clear conception of "non-crossing over in the male." The back-cross tests of the female gave the first "two-point" autosomal map, purple vestigial. The first autosomal "three-point" map was black purple vestigial, completed by the determination of the black-purple cross-over value.

With that most fascinating and difficult subject—the analysis of the relation between the physical chromosome and the process of crossing-over—purple has been intimately connected. The relatively high coincidences obtained in the cases of black purple curved and black purple vestigial soon showed that this relationship in the purple region of the second chromosome is different from the relationship for sections of like map-distance in compared regions of the first chromosome. An explanation of this comparative study should aid in arriving at the cause of the differences.

A study in the case of black purple curved of the change in coincidence accompanying the age variation in crossing-over (Bridges, 1915) led to the tentative conclusion that both changes were mainly due to a lengthening of the average length of the section of chromosome between simultaneous cross-overs, rather than to a change in the freedom of crossing-over. Certain other experiments, notably $\frac{S'}{p_r\, c\, s_p}$, which give information on the age and coincidence changes, have given results that agree better with the first interpretation, though they do not exclude the alternative. The clearest evidence in favor of the internode change is derived from the experiment made by Plough as a control for his temperature-change cultures (*b p, c* B. C., 22°, 2-day interval tube-control). In this experiment the curve for variation in coincidence was the mirror image of the curve of variation in age. The curve of coincidence corresponding to the curve of temperature variation found by Plough seems to be a straight line cutting through the rises and falls of the temperature curve and independent of them. This suggests that the temperature variation is due to a change in a physiological factor different from that involved in the age variation; and that probably it is due to a modification of the coefficient of crossing-over of the chromosome itself.

STRAP (v_g').

(Plate 8, figures 1, 2, 3.)

ORIGIN OF STRAP.

The mutation "strap" was found by Morgan about April 1912, in an experiment involving vestigial flies. The first individual (a female) was regarded simply as a vestigial with extra long wings. The name "strap" was given because of the extreme narrowness of the wings. As in the case of vestigial, they extend laterally from the body, though not quite as nearly at right angles as are the wings of vestigial.

INHERITANCE OF STRAP.

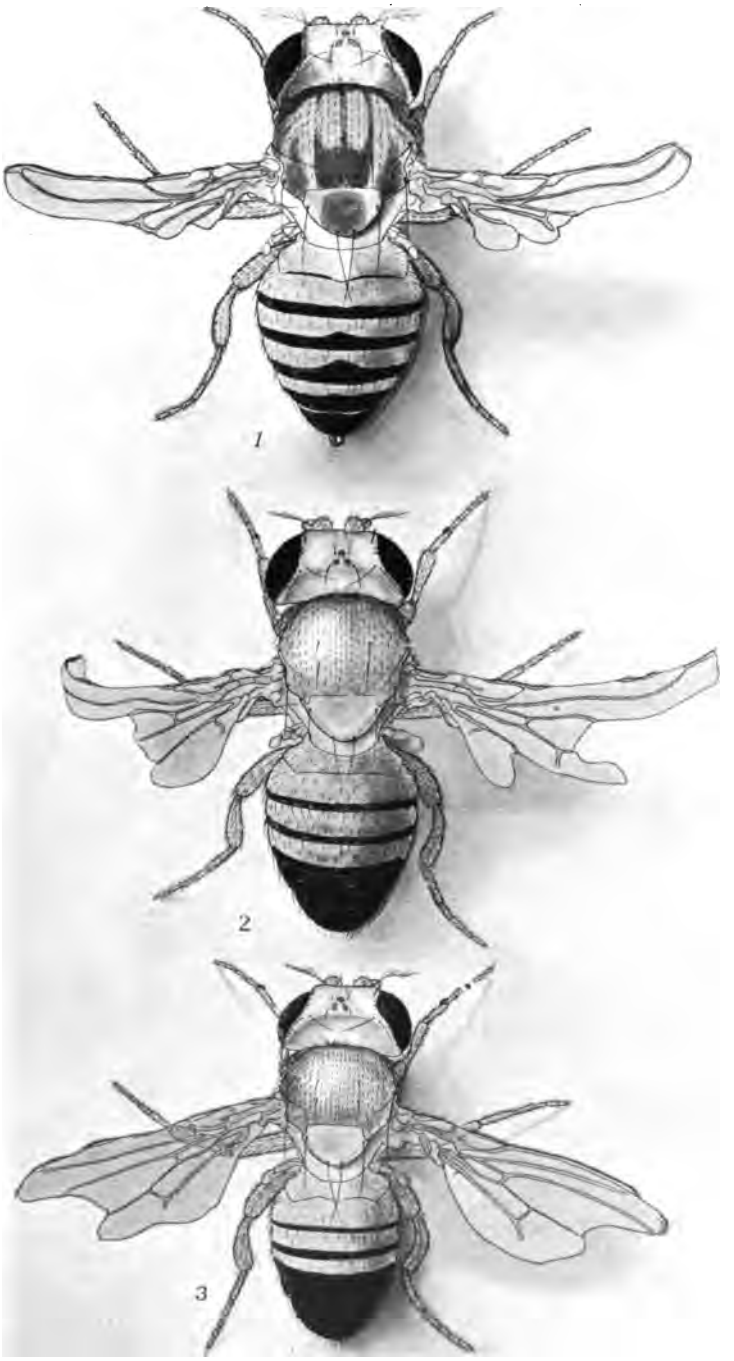
The first female was bred to a vestigial brother and produced in F_1 all vestigial offspring. In F_2, however, the strap-winged type reappeared in about a quarter of the individuals, showing that strap was not simply a "long" vestigial, but that there had been a distinct mutation. That the mutation was not sex-linked was evident from the F_1 result, since the sons of the strap female had failed to show the strap character.

STOCK OF STRAP.

Some of these F_2 strap individuals were inbred and gave a stock practically all of which were "strap." Selection was practiced for many generations to increase the length and narrowness of the wings, but without success further than to establish a homogeneous stock, in which every individual was typically strap.

DESCRIPTION OF STRAP.

While there is considerable variation in the character, the wing is always found to be longer than vestigial. The tip of the wing is narrow and elongated, while the base is broader than vestigial, especially on the inner (rear) margin, so that the whole wing often has a "leg-o'-mutton" appearance. The venation is normal; that is, the bases of all the longitudinal veins are present and usually nearly all of the second longitudinal vein which runs out along the narrow tip either near or at its outer (forward)margin. The rest of the venation is cut away along with the blade of the wing The balancers are affected in an analogous manner, the terminal segment being much reduced, though probably never gone, as it often is in vestigial. A curious correlation noted is that the more of the wing-blade there is present the larger is this terminal segment of the balancer and also the closer to normal is the position of the wing.


1
2
3

CHROMOSOME CARRYING STRAP.

The view of strap prevalent at that time was that it was vestigial modified in the direction of the wild form by a recessive autosomal modifier. On this assumption it was expected that in crosses to wild there would be produced in F_2 vestigials as well as straps, and also some flies having the modifier only. It was problematical what these latter flies should look like, though in general it was expected that they might have longer wings than normal, just as strap was larger than vestigial. In F_2, however, no vestigial appeared and the only certain classes were the wild-type and the strap flies in the usual 3:1 ratio. It is true that a few of the flies were vestigial-like, but none were typical vestigials, and these intermediate forms were regarded as fluctuations of strap.

One of these vestigial-like straps was crossed out to wild and 8 F_2 cultures were raised. Among the 940 F_2 offspring there were only 20 that repeated the vestigial-like appearance; the remainder (175) of the strap flies graded from this shortest type through the usual range of strap.

The F_2 (table 46) from the cross of strap to black gave a typical 2:1:1:0 ratio, which showed that strap was in the second chromosome, as had been concluded from the failure of vestigials to appear in the former F_2. There was here also no apparent crossing-over between vestigial and a possible vestigial modifier responsible for strap.

TABLE 46.—*P_1, strap ♀ × black ♂; F_1 wild-type ♀ + F_1 wild-type ♂.*

Apr. 1, 1914.	Wild-type.		Black.		Strap.		Black strap.	
	♀	♂	♀	♂	♀	♂	♀	♂
55.1.....	133	117	43	56	58	40	0	0
55.2.....	72	68	28	39	25	30	0	0
55.3.....	55	72	28	28	34	28	0	0
55.4.....	70	58	35	23	33	23	0	0
Total..	330	315	134	146	150	131	0	0

It appeared, then, either that strap is vestigial plus a recessive modifier whose locus is very close indeed to that of vestigial, so that no crossing-over was detected in the very extensive F_2 counts, or, more probably, that strap is an allelomorph of vestigial and recessive to both vestigial and the wild-type allelomorph.

Quite recently several rather complicated experiments have been attempted in an effort to distinguish between these two alternatives, but with no decisive result. We believe, however, that the whole trend of the evidence in *Drosophila* is to the effect that types that behave like strap and vestigial are examples of multiple allelomorphism and not of close linkage. It is probable that the vestigial system

now comprises 5 allelomorphs (wild-type, vestigial, strap, antlered, and nick.)

That strap is entirely independent of the third chromosome in its inheritance was demonstrated by an F_2 and a back-cross carried out between strap and pink. The F_2 ratio approximated 9:3:3:1, and the straps that were pink were of the same type as those that were not.

The back-cross test of F_1 males (table 47) gave 1,063 flies, of which 534 were recombinations. This is a percentage of 50.2, where 50.0 was expected, with free assortment between different chromosomes.

TABLE 47.—*P_1, strap ♀ × pink ♂; F_1 wild-type ♀ back-crossed by strap pink ♂.*

June 25, 1913.	Strap.	Pink.	Strap pink.	Wild-type.
M 38.....	44	50	37	56
M 38r.....	60	78	68	92
M 39.....	58	66	54	58
M 39r.....	84	89	83	86
Total...	246	283	242	292

The character strap has never been used in new linkage experiments, since vestigial answers as well in this regard, and the strap wing is not quite large enough to permit the simultaneous use of such other wing and venation characters as curved, arc, plexus, etc.

ARC (*a*).

(Plate 7, figure 4.)

ORIGIN OF ARC.

A stock of flies was being looked over by Bridges in search of individuals with black palpi, which were occasionally produced, when it was noticed that roughly 10 per cent of the flies were showing a new wing-character (culture B 30, May 24, 1912).

DESCRIPTION OF ARC.

This character was called "arc," since the wing was bent downward in an even curve from base to tip, and also from side to side. The margins tend to roll slightly in diagonal lines, so that the wing approaches a diamond-shape. The wing is somewhat broader than normal. The texture of the wing is only slightly thinner than normal. Usually the wings diverge slightly, and occasionally tilt over to the side, giving the appearance of a droop to the outer edge. As far as can be seen, the character is restricted entirely to these wing changes.

STOCK OF ARC.

The character arc had appeared in females as well as males, so that material was on hand to establish a stock. A mass-culture produced a stock that seemed to be pure. Several pair matings made at the same time proved completely fertile, and from one of these a permanent stock was started.

INHERITANCE OF ARC.

In crosses of arc to wild all the F_1 flies were wild-type, showing that arc is recessive. Three mass-cultures and two pairs of F_1 flies gave a total of 2,596 offspring, of which 648 or 24.8 per cent were arcs (table 48).

TABLE 48.—*P_1, arc ♀♀ × wild ♂♂; F_1 wild-type ♀♀ × F_1 wild-type ♂♂.*

Aug. 5, 1912.	Wild-type.	Arc.
B 63.....	528	205
B 64.....	390	126
B 65.....	509	167
B 66.....	272	89
B 67.....	249	61
Total....	1,948	648

EPIDEMIC OF ARCS.

Just as in the cases of purple and of jaunty, there was a short period following the discovery of arc during which arcs appeared and in the most diverse stocks. Within 6 months 9 other appearances of arcs had been recorded by Bridges (table 47). Of these, arc 8 proved to be sex-linked (bow), and at least 2 others were not arc itself, since when bred to arc they gave straight wings. Arcs 7 and 9 were the same as (or at least allelomorphic to) the original arc. Both of these occurred in distinct stocks and were probably independent appearances. Cultures B 66 and B 67 (table 48) are F_2's from arc 7 by wild.

CHROMOSOME CARRYING ARC.

At this time (June 1912) the autosome groups were still very nebulous. The "second" group was slowly condensing around black, but so far as recognized comprised only black, curved, purple, and vestigial, though other mutants had been found which later evidence showed were second chromosome. The key tests had not yet been made which linked all of these into a solid system. The third chromosome group was in far worse plight, being defined simply by pink. It was for this reason that in testing the chromosome of arc some experiments were made that would now be useless. For example, arc was crossed to pink and gave in F_2 a close approach to a 9:3:3:1 ratio (table 49).

The presence of the double recessive arc pink in the F_2 of the "repulsion" cross would now be regarded as conclusive proof that arc was not third-chromosome; but at that time the fact that there is no crossing-over in the male had not yet been discovered, and the above result meant to us either free crossing-over or separate chromosomes. Only further tests could decide which of these alternatives was correct. There were still other reasons for further tests. At this early period it was important to prove to the satisfaction of everybody that if a new mutation showed linkage to any one member of a group

TABLE 49.—P_1, *pink* ♀ × *arc* ♂; F_1 *wild-type* ♀♀ + F_1 *wild-type* ♂♂.

July 11, 1912.	Wild-type.	Arc.	Pink.	Arc pink.
B 46.....	260	104	100	36
B 47.....	112	27	71	21
B 48.....	201	49	58	27
B 49.....	102	31	30	13
Total.....	675	211	259	97

it must show linkage to every member, even though in some cases this linkage be very slight because of the long distance between the loci. Conversely, if a mutant failed to show linkage to one member of a group it was still necessary to show that it would likewise fail to show linkage to other members. For this reason arc had been crossed to maroon at the same time as to pink. It is true that it was not then

TABLE 50.—P_1, *arc* ♀ × *maroon* ♂; *B.C.*, F_1 *wild-type* ♀♀ × *arc maroon* ♂♂.

July 31, 1912.	Arc.	Maroon.	Arc maroon.	Wild-type.
B 60.....	214	209	247	195
B 61.....	230	204	206	211
B 62.....	260	231	266	255
Total.....	704	644	719	661

definitely known that maroon was third-chromosome, but it was seen that these tests would determine to which chromosome maroon belonged. The arc-maroon "repulsion" likewise gave an approach to a 9:3:3:1 ratio. (Culture B 50; + 158, a 54, m_a 41, a m_a 20.)

To make more certain that no appreciable linkage was present, three back-cross cultures were made (table 50).

In the total of 2,728 flies of this arc maroon back-cross there were 1,348, or 49.05 per cent of recombinations, where 50.0 is expected from free assortment.

The independence shown in the arc pink and arc maroon crosses was interpreted to mean that arc was in a separate chromosome from these two and therefore probably in the second chromosome. That this was the case was proved by the result of the cross of arc by black, which produced in F_2 no double-recessive black arc (table 51).

TABLE 51.—P_1, *arc* ♀ × *black* ♂; F_1 *wild-type* ♀♀ × F_1 *wild-type* ♂♂.

July 10, 1912.	Wild-type.	Black.	Arc.	Black arc.
B 42.....	314	130	152	0
B 43.....	134	62	48	0
B 44.....	145	59	45	0
B 45.....	94	51	49	0
B 45.1.....	236	99	93	0
Total.....	923	401	387	0

While this result was accepted as providing that arc was linked to black and was therefore second-chromosome, it was not regarded as proving absolute linkage of black and arc, but merely linkage so close that two cross-over gametes had not chanced to meet. The correct interpretation that the 2 : 1 : 1 : 0 was the result of no crossing-over in the male was not suspected; however, it was considered remarkable that all the autosomal linkages thus far encountered had been so extreme.

LOCUS OF ARC.

In order to conduct a back-cross test of the amount of crossing-over between black and arc it was necessary to obtain the double recessive black arc. By accident the most advantageous method was used, namely, F_2 blacks and F_2 arcs were mated together. Several mass-cultures of this sort were started from B 42 and fortunately black arc flies appeared in F_3. From these a pure stock was made.

The actual back-cross test was not carried out for some months; meanwhile the black vestigial back-crosses had demonstrated that in that case at least there was no crossing-over in the male. It was necessary to test this fact by other cases, since the purple vestigial back-cross had previously given a few apparent cross-overs in the male. "Coupling" back-cross tests of the female and of the male were therefore started at the same time from the mating of black arc male to wild female. The test of the male furnished 306 flies, every one of which was a non-crossover (table 52).

The result of the male test was particularly striking in view of the very free crossing-over shown by the parallel tests of the female (table 53).

In the female tests there was a total of 798 flies, of which 286 or 35.9 per cent were cross-overs.

It was assumed without question that arc was in the same direction from black as were purple and vestigial, the only three genes previously mapped. Arc and vestigial do not make a classifiable combination, so that it was not advisable to test further the locus of arc by means of the vestigial-arc back-cross; but purple and arc are workable and accordingly the purple arc double recessive was made up. By this time, however (April 1913), curved and speck had been mapped, and the position of speck was seen to be close to the assumed position of arc, but even farther away from black, since black speck gave about 49 per cent of crossing-over as against the 36 given by black arc. It was resolved to run a three-point experiment, using speck as well as purple and arc. The first step in this was to get arc and speck together, which proved a troublesome job. The difficulty lay solely in the fact that the loci of these two are so close together that only rarely was one

TABLE 52.—*P_1, black arc ♂ × wild ♀; B. C., F_1 wild-type ♂ × stock black arc ♀ ♀.*

Dec. 18, 1912.	Black arc.	Wild-type.	Black.	Arc.
II 5.....	145	161	0	0

of the F_2 arcs or F_2 specks a cross-over, and the first two attempts failed. The double was finally obtained (October 1913). It was now an easy matter to obtain the purple arc speck triple recessive (F_2 from the cross of purple arc by arc speck).

TABLE 53.—*P_1, black arc ♂ × wild ♀; B. C., F_1 wild-type ♀ × stock black-arc ♂ ♂.*

Dec. 13, 1912.	Black arc.	Wild-type.	Black.	Arc.
C 172.....	122	190	67	84
II 3.....	101	99	69	66
Total.....	223	289	136	150

The P_1 for the back-cross was made by mating a purple female to an arc speck male, which was considered a better type of mating, from the standpoint of viability, than to have all the mutants enter from one parent.

The back-cross (table 54) furnished 2,625 flies, of which 1,431 were non-cross-overs, 1,038 cross-overs between purple and arc, 128 cross-overs between arc and speck, and 28 cross-overs in both regions at once. The distance of arc from purple was found to be greater than first indicated by the black arc value (36), since now 40.6 per cent of crossing-over was observed. This longer distance is in better agree-

ment with the black speck value and with the short arc speck value found.

The coincidence corresponding to these data is 44.2, calculated as follows:

$$\frac{2625 \times 28 \times 100}{(1038 + 28) \times (128 + 28)} = 44.2$$

This coincidence, as compared with the coincidences found for the other cases involving the region around black, purple, vestigial, and curved, were surprisingly low, and suggested that the coincidences involving the right end were different from those involving the part then regarded as the left end, but now regarded as the middle of the chromosome.

There is one other important linkage experiment involving arc, namely, the black arc morula back-crosses with balanced inviability. These crosses, which furnished a total of 6,794 flies (table 84), are treated under the section on morula.

TABLE 54.—P_1, *purple* ♀ × *arc speck* ♂; *B. C.*, F_1 *wild-type* ♀ × *stock purple arc speck* ♂.

Oct. 24, 1914.	p_r / a s_p		p_r a / s_p		p_r / a s_p		p_r a / s_p		Total.
	Purple.	Arc speck.	Purple arc speck.	Wild-type.	Purple speck.	Arc.	Purple arc.	Speck.	
637	67	68	47	56	3	6	3	2	252
638	35	32	29	44	4	2	.....	.....	146
639	89	89	44	59	6	9	.....	3	299
680	36	34	19	37	3	4	1	3	137
681	99	111	66	74	13	5	.....	2	370
682	69	71	52	68	7	6	1	5	279
683	94	77	67	62	9	13	3	1	326
684	118	82	54	60	16	5	.....	.....	335
685	41	34	32	31	2	1	1	2	144
686	92	93	68	69	4	10	1	.....	337
Total	740	691	478	560	67	61	10	18	2,625

TABLE 55.—*Summary of all linkage data involving arc.*

Loci.	Total.	Cross-overs.	P. ct.	Date.	Source.
Black arc..	798	286	35.9	Dec. 13, 1912	Bridges; *b a* × wild B. C.; C 172, II. 3.
	6,794	2,951	43.4	Aug. 4, 1914	Bridges; *b a* m_r balanced B. C.; 364.
	7,592	3,237	42.6		
Purple arc..	2,625	1,066	40.6	Oct. 24, 1914	Bridges; p_r × *a* s_p B. C.; 37–686.
Arc speck...	2,625	156	5.9	Oct. 24, 1914	Bridges; p_r × *a* s_p B. C.; 637–686.
Arc morula.	6,794	534	7.9	Aug. 4, 1914	Bridges; *b a* m_r balanced B. C.; 364.

A summary of the linkage data involving arc is given in table 55. The locus of arc on the basis of these data is 6.7 units to the left of speck, which is its base of reference, or 98.4 units from star.

The calculation of the locus of arc made by Muller (1916) on the basis of relatively few flies agreed with this position.

VALUATION OF ARC.

Arc is of second rank as a working character; its demerit comes from the fact that the character is occasionally simulated in the wings of flies of not-arc stocks. The occurrence of such mimics in a critical experiment involving arc would lead to confusion in the classification and to seemingly impossible results. The opposite error—failure to distinguish the character in flies really arc—occurs rarely or possibly never. In all other respects arc is of first rank; in viability and habit it is excellent; its locus is especially convenient, since it is situated in the gap between curved and speck near the right end of the known chromosome, and since the arc-speck interval of 5.9 is, like black purple, long enough to avoid the high probable errors due to small percentages of crossing-over, but short enough to avoid all danger of double crossing-over within it, and likewise to afford a concise "caliper" region in studies on coincidence or linkage.

GAP.

(Text-figure 76.)

ORIGIN OF GAP.

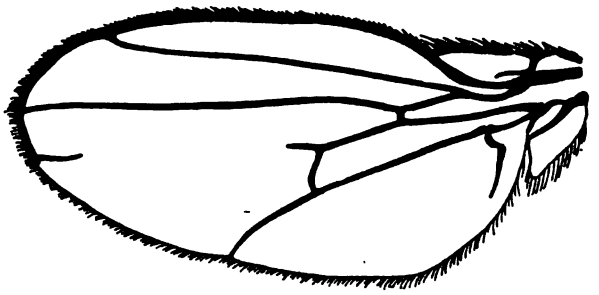
The first appearance of the character called "gap" was in an F_1 mass-culture from the cross of black by arc (July 10, 1912, culture B 42, table 51). Several of the black flies of that culture showed a break or gap in the fourth longitudinal vein between the posterior cross-vein and the wing-tip. This gap varied from nearly all of this section (see fig. 76) to a mere weakening of the vein. The black color, which normally in black flies forms a heavy band on each side of the veins, was likewise absent from this region. From B 42 the stock of black arc was derived, and this stock occasionally showed the gap character. Little attention was paid to it until it turned up again in a cross involving black arc (March 19, 1913).

INHERITANCE OF GAP.

A gap black arc female was then crossed back to a stock black arc male which did not show gap. In F_1 there appeared 27 gap to 55 not-gap offspring (M 37). It was not known whether this denoted that the male had been heterozygous for gap, which is recessive, or that the gap character was dominant. In either case the character gave little promise of usefulness, since obviously some of the flies really gap were

failing to show the character. Such a character can be used, though very inefficiently, by considering those flies which do show the character and disregarding the majority which do not.

That gap is recessive was indicated by the mating carried out when gap reappeared in still another experiment (May 27, 1913). A gap (black arc) female out-crossed to a wild male gave only not-gap offspring (Culture II 38, + ♀51, × ♂63). On the other hand, another such female out-crossed to a black male gave in F_1 38 gap to 65 not-gap offspring (II 41). This seemed to indicate dominance, since the black stock had been examined several times and no gaps had been found. In addition, several of the gap males and females were bred together, and these produced a stock which seemed pure, since in every fly at least a weakening of the vein and color could be seen, while fully 90 per cent showed a distinct and large gap. The stock maintained this condition when run without selection, while at the same time the



TEXT-FIGURE 76.—Gap venation, showing the break in the fourth longitudinal vein.

regular black and black arc stocks failed to show gap when examined occasionally. It now seemed probable that there might be some special relation operative in the case of gap to account for the apparent dominance when crossed to black or black-arc and the recessiveness when crossed to wild. About a year later (July 1914) the cross of gap black arc to black arc was repeated, whereupon the same dominance reappeared in the case of two tests out of the three (table 56). Some of these F_1 gap males were in turn crossed back to black-arc females from stock, with the result that only a very small percentage of the offspring showed gap (table 56). Most of these gaps were females and all showed the character very weakly. This result was in sharp contrast to the F_1 result, where the gap character was well developed, and appeared in fully half the flies, about equally in males and females.

A not-gap F_1 black arc male similarly back-crossed to a black arc female of stock failed to give any gap in 123 flies. This may indicate a genetic difference between the gap and not-gap F_1 flies. An F_2 culture raised from a gap female and a not-gap male showed 35 strongly

developed gaps in a total of 115 flies, or 30 per cent. If the gap F_1 mother had been homozygous for gap and the not-gap father heterozygous, 50 per cent of gap offspring would have been expected. Cultures M 37, II 41, and 397 each gave very nearly a 2 not-gap to 1 gap ratio. Their total was exactly 200 not-gap to 100 gap.

No satisfactory conclusion has been drawn from these data, though on the whole, gap seems to be a recessive character, and there is probably present in the original black-arc stock some special modifier or relationship that makes gap appear in the F_1 of the cross. There seems also to be a sex-limited or sex-linked difference.

The gap stock is still (April 1918) on hand and shows the same condition of the character after 5 years of unselected culture.

TABLE 56.—*P_1, gap black arc ♀ × black arc ♂.*

July 18, 1914.	Black arc ♀.	Black arc ♂.	Gap black arc ♀.	Gap black arc ♂.
304	15	8	13	9
305	14	3	12	10
306	10	19	.....	.....
F_1 gap black arc ♂ × black arc ♀ of stock.				
356	59	56	.....	.....
358	42	48	9	1
359	59	59	7	1
381	125	136	4	.....
382	81	103	3	.....
F_1 not-gap black arc ♂ × black arc ♀ of stock.				
360	55	68	.....	.....
F_1 gap black arc ♀ + F_1 not-gap black arc ♂.				
397	80		35	

CHROMOSOME AND LOCUS OF GAP.

The evidence that gap is second-chromosome consists solely in the persistence with which gap has accompanied black through certain crosses. The fact that a cross-over between black and gap in getting black arc stock retained gap with the black suggests that the locus of gap is to the left of arc and perhaps near curved.

ANTLERED (*vg*^{a}).

(Plate 9.)

ORIGIN OF ANTLERED.

The character "antlered" was found by Morgan about September 1912, and a pure stock was secured. It seems to have originated in an experiment involving vestigial.

DESCRIPTION OF ANTLERED.

The wings of antlered flies are on the average longer than those of strap, often, indeed, being full length. The wing is also broader in the distal portion, so that sometimes it can scarcely be distinguished in form from a rather extreme beaded. Like strap, too, the wings are held out at rather wide angles (about 30° from the axis of the body). A unique feature of antlered is that the long type of wing is quite often folded at the tip (see fig. 2, plate 9).

INHERITANCE OF ANTLERED.

Rather extensive breeding and selection experiments were carried out on this wing, the records of which have been lost. The results, however, were in agreement with some later data, which may be given. Antlered males out-crossed to wild females gave wild-type F_1 offspring. Seventeen mass-cultures of the F_1 flies were bred, giving in F_2 a total of 5,234 flies, of which 1,036 or 19.8 per cent were antlered (August 1915; Morgan, 1915, p. 10). That is, antlered is a simple recessive to wild, and the F_2 ratio was aberrant because of the crowding

TABLE 57.—*P_1, antlered ♂♂ × vestigial ♀♀. F_1 vestigial ♀♀ + F_1 vestigial? ♂♂.*

March 4, 1913.	Vestigial ♀.	Antlered ♀.	Vestigial ♂.	Antlered ♂.
No. 25.....	87	36	37	80
28.....	175	48	96	60
28.1.....	249	38	186	62
30.....	191	13	113	43
31.....	63	42	15	72
33.....	132	12	100	50
34.....	183	64	103	135
35.....	191	64	81	80
Total.....	1,271	317	731	582

that always takes place in mass-cultures. The antlered did not split up in F_2, which shows that antlered is not vestigial plus a modifier unless that modifier is in the second chromosome linked so closely to vestigial that no appreciable crossing-over occurred.

When antlered males were crossed to vestigial females the F_1 flies were not wild-type. They are recorded as being all vestigial (Feb-

ruary 8, 1913; Bridges, p. 35), but this is probably incorrect in view of all other results. No counts were made, or it would probably have been noticed that some at least of the F_1 flies were more like antlered than vestigial.

Eight F_2 mass-cultures were raised (table 57), and these produced a total of 2,901 flies, of which 899 or 31.0 per cent were antlered. On the basis that antlered is completely recessive to vestigial, only 25 per cent of the flies should have been antlered; that is, twice (31−25) or 12 per cent of the vestigial-antlered compounds were more like antlered than vestigial.

An examination of the F_2 counts showed that antlered dominated in the males but not in the females. The antlered males comprised 44.3 per cent of all the males, which means that at least 38.6 per cent

TABLE 58.—P_1, *antlered* ♀ × *vestigial* ♂.

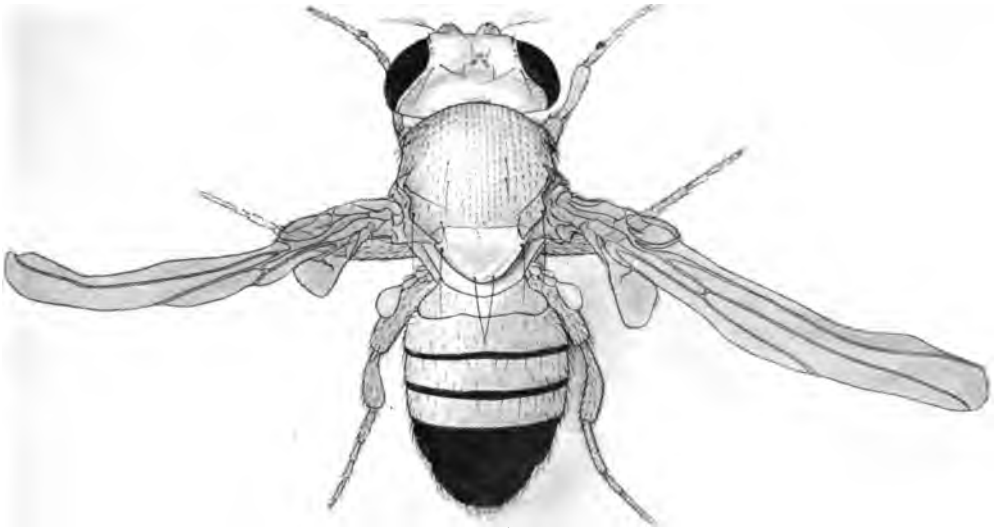
March 26, 1913.	Vestigial ♀.	Antlered ♀.	Vestigial ♂.	Antlered ♂.
39.....	92	.....	57	36
39.1.....	78	.....	28	40
40.....	130	.....	69	25
Total.....	300	.....	154	101
F_1 vestigial ♀♀ + F_1 vestigial ♂♂.				
40.1.....	266	104	187	211
41.1.....	211	66	146	109
41.2.....	98	33	68	52
41.3.....	56	15	31	18
41.4.....	150	42	106	77
42.....	178	61	184	79
Total.....	959	321	724	546

of the vestigial-antlered compound males were antler-like. Among the females only 20 per cent were antler-like, which probably means that the composition in mass-culture had lowered the percentage of antlered females from 25 to 20, just as in the F_2 from antlered by wild the percentage of antlered was only 19.8.

The reciprocal cross (antlered ♀ × vestigial ♂) was started about two months later than the cross just described, and here more attention was paid to the nature of the F_1 flies (table 58).

The relation deduced from the previous F_2 was observed in the new F_1; for while none or only very few of the F_1 females were antlered-like, 36.9 per cent of the males were antlered. These antlered-vestigial compounds were not typical antlered, but were shorter and very much like strap.

Six F_1 mass-cultures gave in F_2 a total of 1,280 females, of which 321 or 25.1 per cent were antlered, and 1,270 males, of which 546 or 43.0



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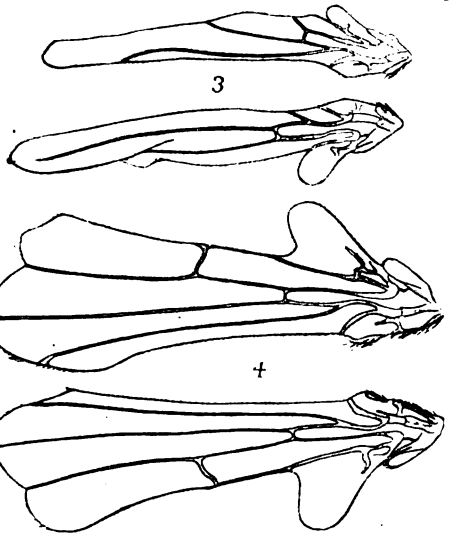

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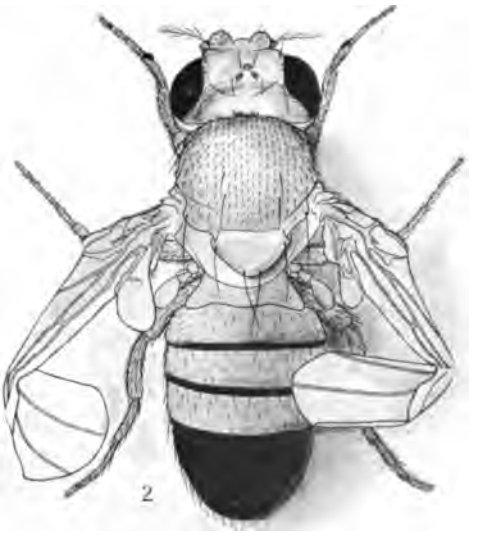

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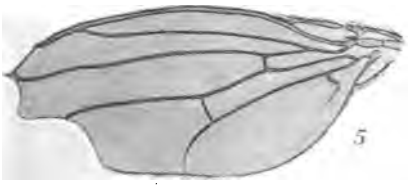

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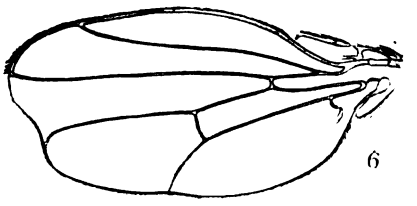


6

per cent were antlered and antlered-like. That is, about 36 per cent of the vestigial-antlered compound males were longer than vestigial (table 58). The percentage of antlered dominance in F_2 (36 per cent) was the same as that observed in F_1 (36.9 per cent).

One other experiment was made in 1913 and repeated in 1915, namely, the cross of antlered male to black vestigial female carried to F_2 (table 59). In the F_1 females there was a slight amount of antlered dominance (6 per cent) and among the males very much more (57 per cent).

TABLE 59.—P_1, *antlered* ♂ × *black vestigial* ♀.

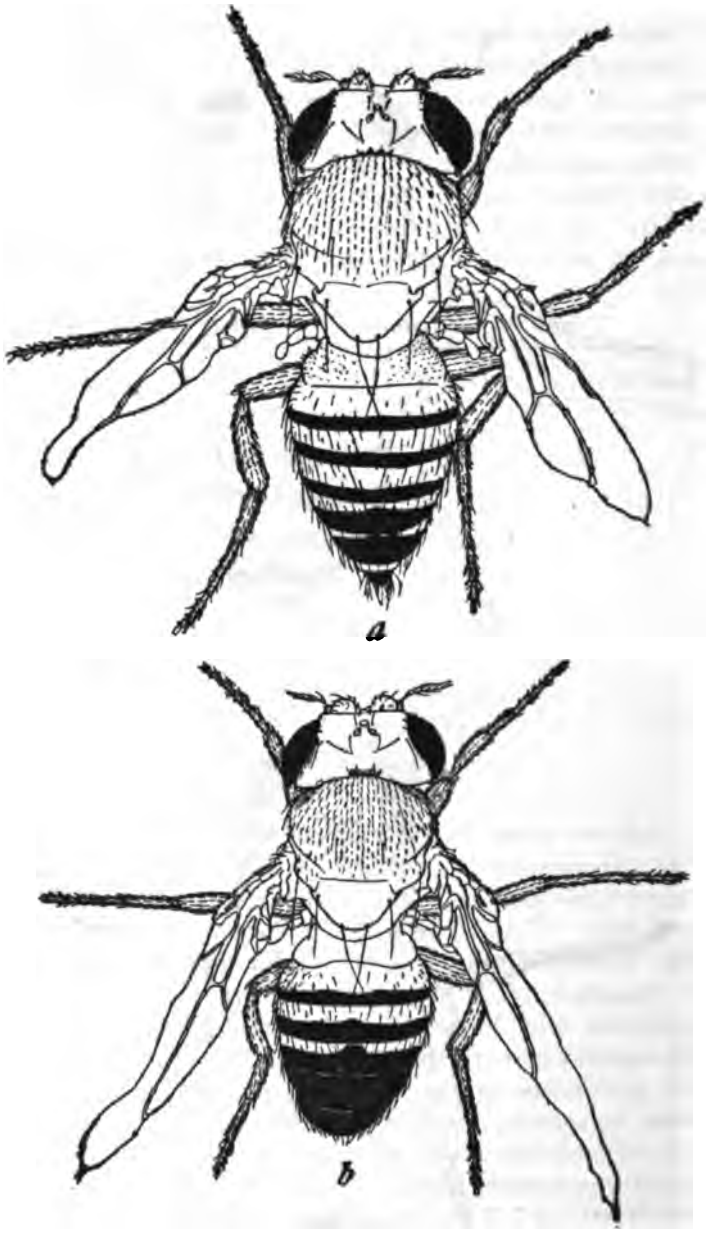
March 22, 1913.	Vestigial ♀.	Antlered ♀.	Vestigial ♂.	Antlered ♂.
II 43	158	10	54	84
II 47	117	7	52	57
Total	275	17	106	141

F_1 vestigial ♀ + F_1 antlered ♂.								
	$b\,v_g$ ♀.	v_g^a ♀.	$b\,v_g^a$ ♀.	v_g ♀.	$b\,v_g$ ♂.	v_g^a ♂.	$b\,v_g^a$ ♂.	v_g ♂.
II 47.1	94	102	2	164	92	145	8	109
1915, 6+17.1	452	596	48	648	371	767	74	269
Total	546	698	50	812	463	912	82	378

The F_2 cultures raised in 1913 agreed closely with the classification of 25 pair cultures made in 1915 by Morgan. Together these F_2 cultures gave 3,941 flies, of which 1,742 or 44.2 per cent were antlered or antlered-like. That is, besides the homozygous antlered, 38.4 per cent of the vestigial-antlered compounds could be separated from the vestigials, though not from the antlered. Among the females the antlered dominance was 23 per cent and among the males 58 per cent, which is in agreement with the 57 per cent observed in the F_1 males.

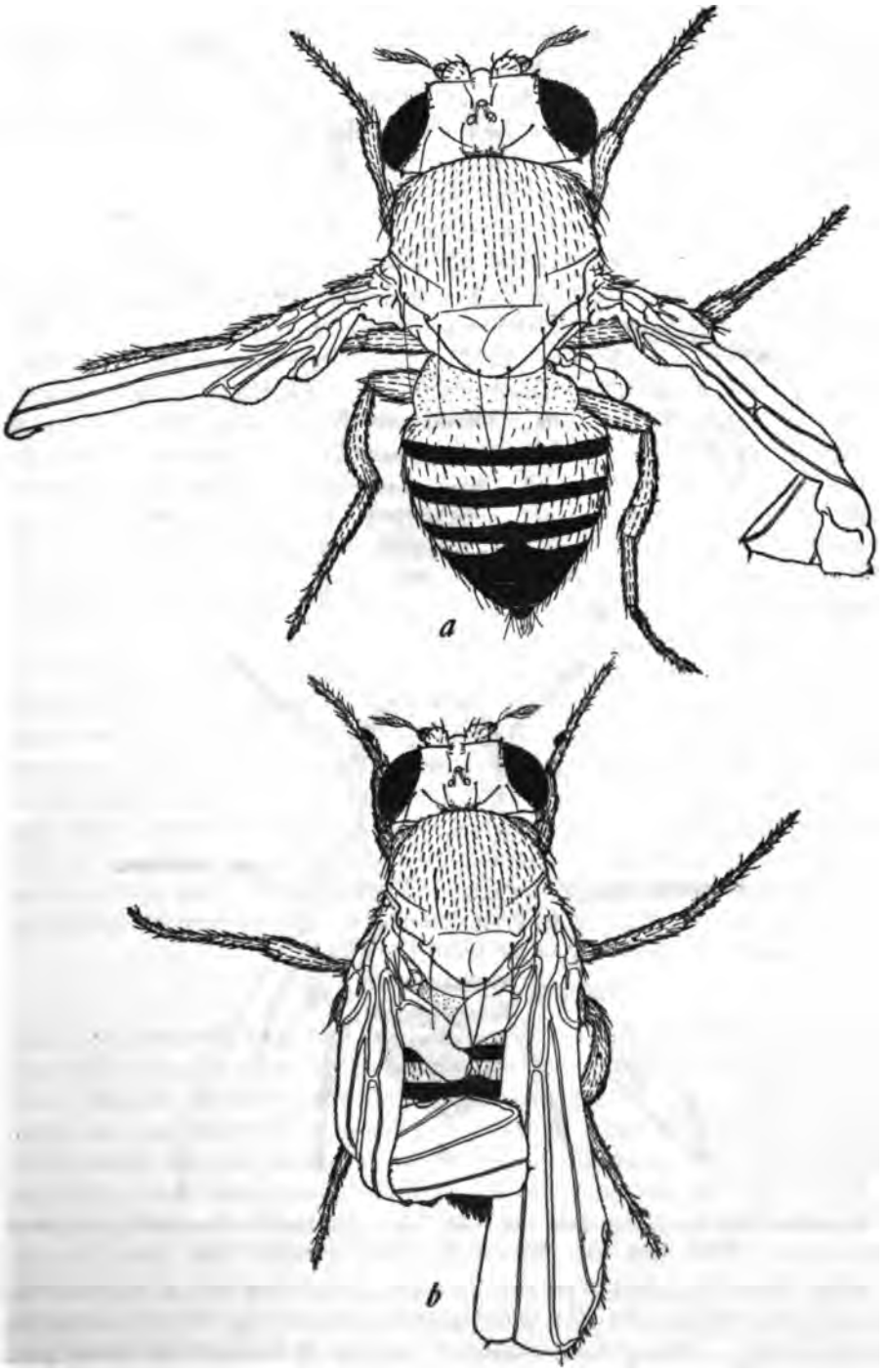
A rough calculation of the amount of crossing-over between black and antlered was made, as follows: There were in the experiment 44.2 per cent of antlered flies where only 25 per cent would have been expected if antlered were a strict recessive. That is, 19.2 per cent (44.2−25) of all the flies (3,941) or 757 flies were due to antlered dominance. There were 132 black antlered flies, all of which were due to antlered dominance and all of which were cross-overs between black and antlered. In the total of 757 comparable flies, 132 or 17.4 per cent were cross-overs, which agrees remarkably with the mapped black vestigial distance of 17.5.

Just as in the case of strap, all the data point to the allelomorphism of antlered to vestigial. The vestigial-antlered compound is in the



TEXT-FIGURE 77.—Vestigial-antlered compounds. Fig. 77*b* shows the compound male, which usually has a longer wing than the corresponding female, fig. 77 *a*.

female indistinguishable from pure vestigial in fully 90 per cent of the flies, and in the remainder is intermediate in type (fig. 77, *a*), and while distinguishable from pure vestigial, grades off toward the pure antlered type. In the males from 40 to 60 per cent of the compounds show enough antlered characteristics to be separable from vestigial, and they approach still closer in type to the pure antlered (fig. 77, *b*).



TEXT-FIGURE 78.—Strap-antlered compounds. Fig. 78*b*, showing the dominance of antlered, is of a male, and 78*a* the less extreme corresponding female.

A similar study has been made by Morgan of the compounds between strap and antlered. In these strap-antlered compounds, both males and females, the antlered allelomorph dominates the strap, so that the flies are in the mass scarcely to be distinguished from antlered (fig. 78, *a* and 78, *b*).

DACHS (*d*).

(Plate 10, figures 1*a* to 1*d*.)

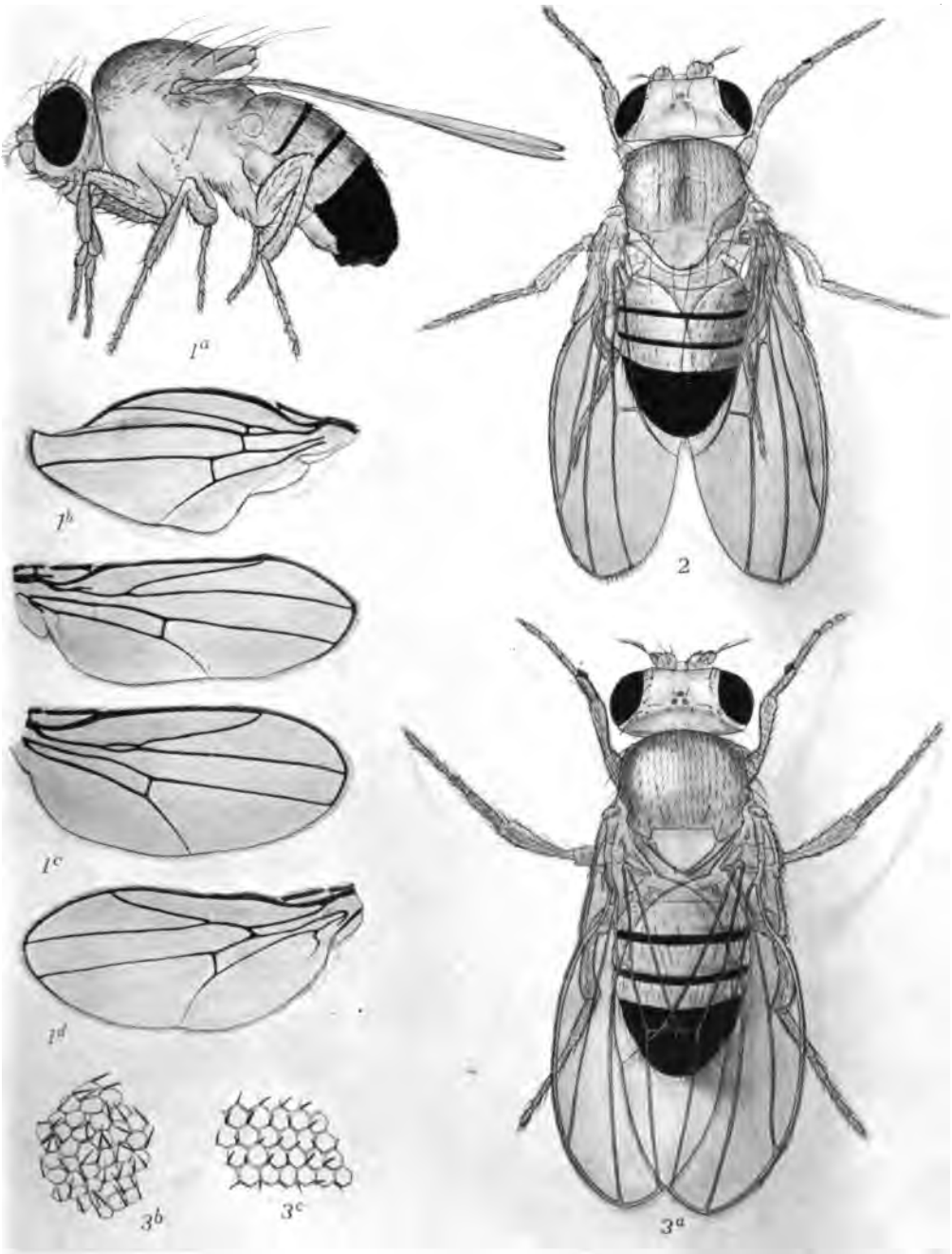
ORIGIN OF DACHS.

The mutant now called dachs had a double origin. Morgan found in certain experiments that the venation of the wing of many of the flies was aberrant (October 1912). This mutant, which he called "shifty," was characterized by a reduction and shifting of the veins in the basal region of the wing. Often the second longitudinal vein is joined to the third near its base or even distal to the anterior cross-vein (plate 10, figs. 1 *b*, 1 *c*, 1 *d*). This anterior cross-vein is itself often absent (figs. 1 *b* and 1 *c*), while in other cases there is an extra cross-vein between the second and third longitudinal veins and placed nearer the base of the wing than the anterior cross-vein (fig. 1 *b*). The wing as a whole is slightly shorter than normal and occasionally is much more reduced, with an accompanying reduction of the venation.

Shortly after this (November 22, 1912) Bridges found in the F_2 of a cross of sable male to wild female 6 females with short legs among about 400 offspring (C146). The F_2 was from a mass-culture, so that there is no significance in finding more than one and less than a quarter of the flies with this character. The fact that all these flies were females also was of no significance, except as an indication that the character was probably autosomal. A pure stock of "dachs," as the mutation was called, was soon extracted from the offspring of the dachs females mated to their wild-type brothers. Some further selection was necessary to eliminate sable from the stock.

DESCRIPTION OF DACHS.

The characteristic feature of dachs is the fact that the tarsus of each leg has only 4 joints instead of the 5 possessed by nearly all other Diptera (plate 10, fig. 1 *a*). These joints are themselves perfectly distinct and normal, except for being slightly shortened. The proximal one has, in the male, the sex-comb typical of the proximal joint of the normal male, so that the omitted joint is one of the more distal ones—perhaps the penultimate. The rest of the leg, especially the tibia, is also shortened. The legs are drawn in close, so that they seem much shorter than they are, and the "dachs" appearance is accentuated. It was soon noticed that the venation was aberrant in the same manner as in "shifty" flies, and an examination of the shifty stock showed that all of them had four-jointed tarsi. In fact, the two stocks were probably identical. Whether they were of single origin (from the wild


1a
1b
1c
1d
2
3a
3b
3c

stock?) or whether there were two independent mutations is uncertain. Both occurrences have contributed to similar "epidemics of mutation" in the cases of purple, jaunty, arc, etc.

CHROMOSOME CARRYING DACHS.

Since it had become apparent while pure stock was being extracted that dachs was recessive and not sex-linked, we proceeded directly to tests of its autosome group. A dachs male was out-crossed to a black female, and from a pair of the wild-type F_1 flies an F_2 culture was raised (table 60).

TABLE 60.—P_1, *dachs* ♂ × *black* ♀. F_1 *wild-type* ♂ × F_1 *wild-type* ♀.

Jan. 7, 1913.	Wild-type.	Dachs.	Black.	Dachs black.
II 4.....	186	71	93	0

The F_2 flies were in the 2:1:1:0 ratio, which had become recognized as the typical result for two recessives in the same autosome crossed to each other and carried to F_2 ("repulsion" F_2). This ratio is the necessary result of the absence of crossing-over in the male and is independent of the amount of crossing-over in the female.

A similar cross of dachs by curved likewise gave no double recessives in F_2 (table 61). The reason in this case for crossing to two different recessives in the same chromosome was to locate dachs more speedily

TABLE 61.—P_1, *dachs* ♀♀ × *curved* ♂♂. F_1 *wild-type* ♀♀ + F_1 *wild-type* ♂♂.

Jan. 6, 1913.	Wild-type.	Dachs.	Curved.	Dachs curved.
II 8.....	512	169	245	0
II 10.....	166	47	89	0
II 13.....	204	41	96	0
Total.....	882	257	430	0

by working out simultaneously two cross-over values. However, the dachs curved cross was not carried to the back-cross stage, because at that time the locus of curved was itself not sufficiently well established and secondarily because dachs seemed poorly viable in the dachs curved F_3.

From the dachs × black F_2, dachs and blacks were crossed *en masse*, and in F_3, a few dachs black double recessive were secured. One of these was crossed to a wild male as the P_1 for the proposed crosses, and the rest were mated together to supply a dachs black stock to be used in the back-cross tests.

Three F_2 mass-cultures were raised (table 62), from which a rough calculation of the amount of crossing-over was made, as follows: The class dachs black is a non-cross-over simple class. Each of the two complementary classes dachs and black is a cross-over class. The wild-type class is composite, including the wild-type non-cross-over class complementary to the dachs black class, and also all the flies coming

TABLE 62.—P_1, *dachs black* ♀ × *wild* ♂.

F_1 wild-type ♀♀ + F_1 wild-type ♂♂.				
March 18, 1913.	Dachs black.	Wild-type.	Dachs.	Black.
II 34.....	113	403	34	32
II 35.....	58	263	25	20
II 36.....	85	339	28	24
Total.....	256	1,005	87	76
B. C., F_1 wild-type ♂ × dachs black ♀ from stock.				
April 1, 1913.	Dachs black.	Wild-type.	Dachs.	Black.
II 37.....	72	98	0	0
B. C. F_1 wild-type ♀ × dachs black ♂ from stock.				
June 30, 1913.	Dachs black.	Wild-type.	Dachs.	Black.
II 40.....	64	91	9	12
II 56.....	47	112	6	25
II 57.....	52	75	9	15
II 91.....	13	53	3	9
II 94.....	14	56	4	9
II 98.....	27	38	9	16
II 98r.....	58	70	15	22
Total.....	275	495	55	108

from that half of the sperm which carried the wild-type chromosome, for which reason it can not be used in a simple calculation. The average of the dachs and the black classes $\left(\frac{87+76}{2}=82\right)$ was used as the cross-over class to compare with the 256 dachs black non-cross-overs. The percentage of crossing-over thus calculated was 24.3, and this was temporarily taken to be the dachs black distance. The position of dachs was assumed to be to the right of black, since all the other mutants thus far found were in that direction.

A male test was made by back-crossing an F_1 male by a dachs black female (table 62). There were no cross-overs in a total of 170 flies.

The back-cross tests of the female (table 62), somewhat delayed, gave a total of 933 flies, of which 163 were cross-overs. The percentage of crossing-over calculated from these back-cross results was 17.7—a much more dependable figure than that derived from the F_2.

TABLE 63.—P_1, *dachs black vestigial* ♂♂ × *wild* ♀♀. F_1 *wild-type* ♀ × *dachs black vestigial* ♂♂ *from stock.*

Dec. 10, 1913.	d b v_g		d | b v_g		d b | v_g		d | v_g | b		Total.
	Dachs black vestigial.	Wild-type.	Dachs.	Black vestigial.	Dachs black.	Vestigial.	Dachs vestigial.	Black.	
II 114.....	99	116	25	19	23	20	5	2	309
II 115.....	117	133	30	34	18	20	4	4	360
II 116.....	95	100	16	14	19	15	4	4	267
II 117.....	85	89	22	21	23	22	3	2	267
II 119.....	75	67	11	16	9	12	1	1	192
II 138.....	68	102	13	18	14	25	2	1	243
Total....	539	607	117	122	106	114	19	14	1,638

One more experiment was needed to finish the determination of the locus of dachs. The locus of dachs was known to be about 18 units from that of black, and while it had been assumed that dachs lies to the right of black, this could only be established by finding the cross-over value for dachs and another of the mutants whose locus was known. Vestigial was chosen for this test and preparations were made to carry out a balanced triple back-cross. The triple-recessive dachs black vestigial was obtained in F_2 from the cross of dachs black to black vestigial.

TABLE 64.—P_1, *dachs* ♂♂ × *black vestigial* ♀♀. F_1 *wild-type* ♀ × *dachs black vestigial* ♂♂ *from stock.*

Dec. 11, 1913.	d b v_g		d | b v_g		d | v_g b		d b | v_g		Total.
	Dachs.	Black vestigial.	Dachs black vestigial.	Wild-type.	Dachs vestigial.	Black.	Dachs black.	Vestigial.	
II 120.....	88	135	12	29	22	26	3	3	318
II 121.....	51	79	12	15	8	18	1	.....	184
II 122.....	81	98	10	36	7	21	3	5	261
II 123.....	87	110	16	43	23	19	3	5	306
II 124.....	93	147	20	36	15	27	1	5	344
II 126.....	74	70	12	16	18	17	2	.....	209
Total.....	474	639	82	175	93	128	13	18	1,622

The triple back-cross was made to the extent of about 1,600 flies in three of the four possible ways (tables 63, 64, and 65) and gave a grand total of 4,892 flies (table 66) of which 3,329 were non-cross-overs, 757 were cross-overs between dachs and black, 689 were cross-overs between black and vestigial, and 117 were double cross-overs.

As soon as the first flies from the triple back-cross had begun to hatch it became evident that the locus of dachs is to the left of black and not to the right. The discovery that streak, a dominant, was so far to the left of black that it gave very free crossing-over had just been made and had led to the most extensive recasting that any of our maps had been subjected to. The location of dachs in the middle of this long gap was therefore very welcome.

TABLE 65.—*P_1, dachs black ♂♂ × vestigial ♀♀. F_1 wild type ♀ dachs black-vestigial ♂♂ from stock.*

Dec. 11, 1913.	d b / v_g		d / v_g b		d b / v_g		d / b v_g		Total.
	Dachs black.	Vestigial.	Dachs vestigial.	Black.	Dachs black vestigial.	Wild-type.	Dachs.	Black vestigial.	
II 127.....	69	96	28	29	26	24	3	5	280
II 128.....	111	109	23	20	32	31	4	6	336
II 129.....	78	82	13	19	10	16	3	2	223
II 130.....	101	141	30	34	18	31	4	8	367
II 131.....	109	174	26	39	27	33	12	6	426
Total.....	468	602	120	141	113	135	26	27	1,632

The total amount of crossing-over between dachs and black as calculated from this balanced experiment was 17.9 units, which agreed with the 17.7 units found in the simple dachs black back-cross.

TABLE 66.—*Linkage of dachs black and vestigial with balanced inviability.*

Dec. 10, 1913.	———	—|——	——|—	—|—|—	Total.	Cross-over values.		
						d b	b v_g	d v_g
d b v_g.....	1,146	239	220	33	1,638	16.6	15.5	28.1
d / b v_g.....	1,113	257	221	31	1,622	17.8	15.5	29.5
d b / v_g.....	1,070	261	248	53	1,632	19.2	18.4	31.2
Total.....	3,329	757	689	117	4,892	17.9	16.5	29.7

Table 67 gives the summary of the crossing-over data including dachs. The calculation of the locus of dachs on the basis of all the data is 17.5 units to the left of black, or referred to star as the zero-point at 29.0.

VALUATION OF DACHS.

The usefulness of dachs is limited only by its rather poor and erratic viability. In many experiments the viability of dachs is up to par, but in combination with certain other characters it has been unsatisfactory; thus the stock called "π" (d b p_r c p_x s_p) is poorly viable and almost useless, while the "$\pi-$" stock, which differs only in the omission of

TABLE. 67.—*Summary of the cross-over data involving dachs.*

Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Star dachs....	96	31	32.3	Sept. 12, 1915	Bridges	d_i; $\frac{S'}{d}$ F_2, dachs flies; 2141–2216.
	152	53	34.8	Sept. 12, 1915	Do.	Idem, not-dachs flies.
	1,617	425	26.3	Sept. 15, 1915	Do.	S'; $\frac{S'}{d}$ B.C.; 2146–2305.
	369	112	30.4	Oct. 6, 1915	Do.	d_i; $\frac{S'}{d_i}$ F_2; 2217–2659.
	211	57	27.1	Nov. 18, 1915	Do.	d_i; $\frac{S'}{d_i}$ B. C.; 2460.
	1,027	271	26.4	Aug. 24, 1916	Do.	S'; $\frac{S' \quad p_r}{S_k \; d}$ B. C.; 4999–5110.
	3,472	949	27.3			
Streak dachs..	462	45	9.7	May —, 1914	Muller	Am. Nat., 1916, p. 422.
	396	64	16.2	Aug. 24, 1916	Bridges	S'; $\frac{S' \quad p_r}{S_k \; d}$ B. C.; 4999–5110.
	858	109	12.7			
Dachs black...	338	82	24.3	Mar. 18, 1913	Bridges	*d*; *d b*. F_2; II 34–II 36.
	933	163	17.5	June 30, 1913	Do.	*d*; *d b* B. C.; II 40–II 98r.
	4,892	874	17.9	Dec. 10, 1913	Do.	*d*; *d b* v_g balanced B. C.; II 114–II 138.
	462	77	16.7	May —, 1914	Muller	Am. Nat., 1916, p. 422.
	6,725	1,196	17.8			
Dachs purple..	462	97	21.0	May —, 1914	Muller	Am. Nat., 1916, p. 422.
	1,027	196	19.1	Aug. 24, 1916	Bridges	S'; $\frac{S' \quad p_r}{S_k \; d}$ B. C.; 4999–5110.
	1,489	293	19.7			
Dachs vestigial.	4,892	1,456	29.7	Dec. 20, 1913	Bridges	*d*; *d b* v_g balanced B. C.; II 114–II 138.
	462	129	27.9	May —, 1914	Muller	Am. Nat., 1916, p. 422.
	5,354	1,585	29.6			
Dachs curved .	462	145	31.4	May —, 1914	Muller	Do.
Dachs speck...	462	231	50.0	May —, 1914	Do.	Do.
Dachs balloon.	462	231	50.0	May —, 1914	Do.	Do.

dachs, is entirely normal in viability. In all other respects dachs is of first rank. Since the character used in classification is the number of joints of the tarsus, there is no masking effect possible with any of the other second-chromosome mutants. The recessiveness of dachs is complete, its identification is perfect, and the separations are easy and rapid.

The locus of dachs is such that it is the most important connecting-link between the left end of the chromosome and the securely established and well-mapped region from black to the right end of the chromosome.

STREAK (S_k).

(Plate 5, figure 5, and Plate 10, figure 2.)

ORIGIN OF STREAK.

In a stock culture from a pair of flies with the mutant called "lop-wing" (culture C 149, November 27, 1912), Bridges found a single female which had a prominent broad, dark streak down the middle of the thorax.

STOCK OF STREAK.

This female (non-virgin) was mated to several of her brothers and produced many streaks among the offspring. It was assumed that the character was recessive and that some of the brothers had been heterozygous. No F_1 counts were made and not much attention was paid to the character.

Several of the streak individuals were mated together to provide stock. In this F_2 culture somewhat more than half (no counts) of the offspring were streak where all had been expected to be streak. This was thought to indicate a "poor" character, which, like truncate, club (wings), and others, shows in a variable proportion of the flies of the same genetic constitution. The stock was carried on in this way for two more generations, when it was decided to throw it away as being too poor to repay further labor. This would have been done had not Morgan seen in this character a bearing on a selection problem which he had been carrying out for over two years on the thorax pattern of "with" flies. In the course of selections for a still darker pattern three notable successes had been obtained, all of which turned out to be simply new mutations (speck, olive, and band) which had occurred in the selected stocks, but which gave no further variability or progress when once the stocks were pure for them. In streak there was an example of a dark thorax character which closely resembled in pattern the darkest of the long selected "bands," though not as dense in pigmentation, but which had arisen entirely independent of any selection whatsoever.

Later the trident mutant "trefoil" (plate 5, fig. 6) likewise arose independent of selection. These independent mutations and the fact that during this same period over a hundred other mutations affecting every part of the body had appeared in *Drosophila*, left no basis whatever for the supposition that the selection had had any effect whatever on either the frequency or the nature of the mutations, or that any other process, aside from the production of three definite mutations, had contributed to the success of the selection.

Morgan selected the streak stock for about six months, though not very vigorously, without increasing the intensity of the pattern or the frequency of the streak individuals.

DESCRIPTION OF STREAK.

The principal characteristic of streak flies is the band of pigment along the thorax and scutellum. This band seems to be rather deep-lying, and is possibly situated in a different layer from that in which the other pigment characters develop. There is considerable variation in both the intensity and the extent of the dark color. In its greatest development it is a solid band, like that of colored figure 5, filling in the entire region between the dorso-central bristles and extending over the entire scutellum. In less-developed types the weakening starts in the region ahead of the dorso-central bristles and is most pronounced between the prongs of the trident pattern, so that an appearance much like typical band is given. (For figures of "with," band, and trefoil, see Mechanism of Mendelian Heredity, p. 206).

The intensity of color is never very great and the color may nearly vanish. However, there are other accessory characteristics that aid in the classification. Chief of these is a flattening of the thorax and the appearance of bubbles. Both of these effects seem to be due to an ill development of the underlying muscles. There appear to be present in the thorax large spaces or sinuses filled only with blood and large bubbles. Where there are no bubbles present this condition is not so easy to distinguish, though it may sometimes be made out by slightly pressing the thorax. The wings are apt to droop and to diverge slightly, probably also on account of the muscular condition.

DOMINANCE AND LETHAL EFFECT OF STREAK, PARALLEL TO YELLOW MOUSE.

The occurrence of the mutation as a single individual—a female—in a pair culture, its immediate reappearance in about half the F_1 flies after crossing to normal males, and the failure of these F_1 flies to breed

TABLE 68.—P_1, *streak* ♀ × *wild* ♂.

July 22, 1914.	Streak ♀.	Streak ♂.	Wild-type ♀.	Wild-type ♂.
336.....	23	22	25	28
391.....	53	69	69	64
393.....	37	37	48	43
394.....	76	68	64	60
Total.....	189	196	206	195

true, found an explanation (rather delayed) in the assumption that streak was an autosomal dominant. Moreover, the fact that the stock could not be made to breed true (continually producing at least a third of the offspring wild-type) and that repeated pair matings as well gave this same result, led to the assumption that homozygous individuals were not produced. This case was seen to be a parallel to

the well-known case of the yellow mouse, and was the first of many to be found in *Drosophila*, where a homozygous dominant is lethal.

Outcrosses of streak by wild gave in F_1 streaks as approximately half of the flies. The records of these early out-crosses were lost (notebook S II), but similar out-crosses made later illustrate the fact as well (table 68).

CHROMOSOME CARRYING STREAK.

The next task was to determine in which chromosome the gene for streak is located. This was done by back-cross tests of the male for black (II chromosome) and pink (III chromosome).

TABLE 69.—*P_1, streak ♀ × pink ♂; F_1 streak ♂ × pink ♀ of stock.*

July 7, 1913.	Streak.	Pink.	Streak pink.	Wild-type.
D 7.....	90	133	71	117
D 7r.....	65	50	77	64
Total.....	155	183	148	181

Streak males heterozygous for pink were produced from the mating of streak female by pink male. Two of these F_1 males were back-crossed to pink females and produced a total of 667 offspring, 329 of which were recombinations (table 69). The presence of the streak-pink and the wild-type flies as 49.3 per cent of the whole proved that streak was not in the third chromosome, since independent assortment was demonstrated.

TABLE 70.—*P_1, streak ♀ × black ♂; F_1 streak ♂ × black ♀ of stock.*

July 7, 1913.	Streak.	Black.	Streak black.	Wild-type.
D 5.....	19	21	0	0

In the back-cross test of the streak male heterozygous for black no recombination occurred. Every one of the 19 not-black flies was distinctly streak, and likewise none of the 21 black flies showed a trace of streak (table 70). This result was due to the fact that the locus of streak is in the second chromosome and the lack of crossing-over in the male.

LOCUS OF STREAK.

Immediately following the appearance of the first flies in the preceding back-cross tests of the male, a test of the locus of streak was made by means of the mutant morula, which had itself just been mapped at the right end of the second chromosome.

Back-cross tests of F_1 streak females, from the cross of streak females by morula males, gave a total of 876 flies, of which 405 or 46.2 per cent were cross-overs (table 71).

This very free crossing-over between streak and morula indicated that streak was far away from the right end of the chromosome, where the gene for morula had been located. It was thought that the locus of streak was in the neighborhood of black (which was at that time considered the left end of the chromosome) or purple, which was not far from black. It did not seem probable that an accurate classification of streak and black could be made at the same time, so purple was used instead.

TABLE 71.—P_1, *streak* ♀ × *morula* ♂; F_1 *streak* ♀ × *morula* ♂ *of stock.*

Aug. 24, 1913.	Streak.	Morula.	Streak-morula.	Wild-type.
II 64.....	11	8	4	6
II 82.....	50	47	40	31
II 92.....	55	55	44	61
II 97.....	124	121	130	89
Total.....	240	231	218	187

A three-locus experiment partly balanced for inviability was carried out. A streak female was out-crossed to a purple curved male and F_1 streak females were back-crossed by purple curved males. Since streak is a dominant, the triple recessive not-streak purple curved was a double mutant form, which was a great saving of labor over the ordinary case, in which the triple mutant multiple recessive has to be made up.

TABLE 72.—P_1, *streak* ♀ × *purple curved* ♂; *B.C.*, F_1 *streak* ♀ × *purple curved* ♂ *of stock.*

Nov. 6, 1913.	S_k / p_r c		S_k | p_r c		S_k c / p_r		S_k | p_r / c	
	Streak.	Purple curved.	Streak purple curved.	Wild-type.	Streak curved.	Purple.	Streak purple.	Curved.
II 103.....	82	81	45	49	31	28	9	12
II 104.....	83	91	57	55	27	18	14	19
II 110.....	46	52	18	23	14	9	8	7
Total.....	211	224	120	127	72	55	31	38

The result of the back-cross (table 72) was surprising, since it upset the ill-founded notion that black was at the left end of the second chromosome. The cross-over values (streak purple = 36.0, purple curved 24.5, streak curved 45.7) and the double cross-over classes (streak purple *versus* curved) showed that streak was fully 40 units to the left of purple (allowing for double crossing-over). No trouble in classifying streak was met in this experiment, so that all of the flies are available for the calculation.

From the streak purple and the curved flies that appeared in the back-cross just described a P_1 mating was made for the second type of

back-cross (table 73). This second back-cross gave linkage results which differed only very slightly from those of the first, and in such a way that by combining the two sets of data the deviations of one tend to balance those of the other and more nearly correct values can be calculated.

TABLE 73.—*P_1, streak purple ♀ × curved ♂; B.C., F_1 streak ♀ × purple curved ♂ of stock.*

Dec. 18, 1913.	S_k p_r / c		S_k / c / p_r		S_k p_r / c		S_k / p_r / c	
	Streak purple.	Curved.	Streak curved.	Purple.	Streak purple curved.	Wild-type.	Streak.	Purple curved.
II 136.....	66	58	23	33	10	11	7	5
II 137.....	29	27	12	16	3	7	7	2
13.....	26	27	8	12	5	10	2	4
15.....	24	45	25	29	9	14	5	5
16.....	54	53	22	32	12	14	8	8
23.....	24	16	14	8	6	2	1	3
24.....	23	24	13	7	5	9	2	3
Total.....	246	250	117	137	50	67	32	30

The combined data gave 1,807 individuals, of which 931 were non-cross-overs, 501 cross-overs between streak and purple, 244 cross-overs between purple and curved, and 131 double cross-overs. The cross-over values calculated from this distribution are streak purple 35.0 per cent, purple curved 20.7 per cent, and streak curved, 41.2 per cent.

The coincidence from the first back-cross was 98.0 and from the second 102.0. The coincidence from the combined data was 99.8

$$\left(\frac{1807\times131\times100}{632\times375}=99.8\right)$$

For a section of this great length and involving this particular region the net result was that the occurrence of a cross-over in one section was without effect upon the occurrence of a cross-over in the other section.

The locus of streak as calculated from the above data, making allowance for the probable amount of double crossing-over, was 40.6 to the left of purple or 34.7 units to the left of black, which was the locus farthest to the left of those previously determined. This great gap was almost immediately filled by the mapping of dachs at 17.9 units to the left of black, or almost exactly midway between the two. This position of dachs offered a new base of reference for streak and one much more dependable than the remote purple. The data on which a direct determination of the streak dachs distance was made is included in the section on star, for by means of a quadruple back-cross involving star, streak, dachs, and purple the loci of both star and streak were linked up with the portions already mapped. The streak dachs interval was found by this experiment to be about 16.2 units.

The calculation of the locus of streak on the basis of all the available data places it at 13.6 units to the left of dachs and 15.4 units to the right of star, star being the zero-point for the chromosome.

A summary of the linkage data directly involving streak is given in table 74.

TABLE 74.—*Summary of cross-over data involving streak.*

Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Star streak.....	396	63	15.9	Aug. 24, 1916	Bridges	S'; $\frac{S' \quad p_r}{S_k \; d}$ B. C.; S_k flies only; 4999–5110.
Streak dachs....	462	45	9.7	May —, 1914	Muller	Am. Nat., 1916, p. 422.
	396	64	16.2	Aug. 24, 1916	Bridges	S'; $\frac{S' \quad p_r}{S_k \; d}$ B. C. Sk' flies only; 4999–5110.
	858	109	12.7			
Streak black.....	462	120	26.0	May —, 1914	Muller	Am. Nat., 1916, p. 422.
Streak purple....	1,807	632	35.0	Nov. 6, 1913	Bridges	S_k; S_k p_r c balanced B. C.; II 103–II 124.
	462	137	29.7	May —, 1914	Muller	Am. Nat., 1916, p. 422.
	396	114	28.8	Aug. 24, 1916	Bridges	S'; $\frac{S' \quad p_r}{S_k \; d}$ B. C.; S_k flies only; 4999–5110.
	2,665	883	33.1			
Streak vestigial..	462	164	35.5	May —, 1914	Muller	Am. Nat., 1916, p. 422.
Streak curved...	1,807	745	41.2	Nov. 6, 1913	Bridges	S_k; S_k p_r c balanced B. C.; II 103–124.
	462	178	38.5	May —, 1914	Muller	Am. Nat., 1916, p. 422.
	2,269	923	40.7			
Streak blistered..	11	5	45.0	Feb. 23, 1914	Bridges	b_s; $\frac{S_k}{b_s}$ B. C.; 69.
Streak speck.....	462	242	52.3	May —, 1914	Muller	Am. Nat., 1916, p. 422.
Streak balloon...	462	242	52.3	May —, 1914	Do.	Do.
Streak morula...	876	405	46.3	Aug. 24, 1913	Bridges	S_k; $\frac{S_k}{m_r}$ B. C.; II 64–II 97.

VALUATION OF STREAK.

There is only one drawback—but that one very serious—to the usefulness of streak, namely, the difficulty of separating all the streaks from the wild-type. In most of the experiments conducted by Bridges this difficulty was not great enough to impair the accuracy of the result. However, a streak dachs back-cross was attempted and abandoned, and in the quadruple back-cross $\left(\frac{S' \quad p_r}{S_k \; d}\right)$ the separation is not complete in all cultures. In these cultures the first separation performed was that of the streak from the not-streak, without regard to the other

character. Among the streaks the other mutant characters should be distributed in the same ratio as among all the flies, so that tolerably accurate calculations could be made using streak flies only, but such experiments are inefficient. In the "progeny test" experiment of Muller this difficulty was entirely avoided, since the easily determined presence or the absence of streak from a progeny-test culture was all that was required to classify each parent. Streak is not a character that can be successfully handled without quite extensive experience, and even under the best of conditions there is chance of error.

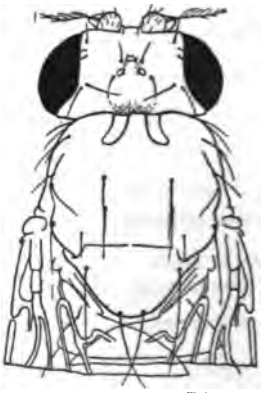
In favor of streak is its location, which is very important as the link between star and the rest of the chromosomes. A favorable location far more than doubles the usefulness of a character, other things being equal. In viability and other features streak is satisfactory.

COMMA.

(Text-figure 79.)

ORIGIN OF COMMA.

In one of the F_2 cultures from the cross of dachs by pink, there appeared a mutant character called "comma" (culture II 9, February 5, 1913), which consists of a pair of chitinous thickenings on the anterior dorsal part of the thorax (fig. 79). In shape these thickenings are like a pair of commas, lying back to back, with the blunt tails pointing posteriorly, and depressed below the general level of the surface. This character was confined very largely to the females, of which about 20 per cent showed the character; a few of the males also were commas.



TEXT-FIGURE 79.—Diagram of "comma."

From the frequency of the commas it was concluded that the character was an autosomal recessive, which was either very inviable or, more probably, failed to show in all those flies which were homozygous. In either case the character was markedly sex-limited in the sense that under like conditions far fewer of the males than of the females showed the character.

CHROMOSOME CARRYING COMMA.

In the F_2 of the dachs pink cross, the commas seemed to be distributed at random among the pinks and the wild-types, while none were seen among the dachs. This was interpreted as meaning that the locus is in the second chromosome. To test this point, commas were outcrossed to pink of the third chromosome and to vestigial of the second chromosome and an F_2 mass-culture was raised in each case (tables

75 and 76). Two or three out-crosses of the pink comma flies were also attempted to secure P_1 for a back-cross, but these failed.

The F_2 of the comma by pink gave only 8 commas, all females, among 440 flies. Of these 8, 3 were pink commas, so that comma was proved to be not third-chromosome. The fewness of the commas was partly due to the crowding of the mass-cultures, but more to the fact that the character fails to show on all the flies that are genetically commas.

TABLE 75.—P_1, *comma* ♀ × *pink* ♂. F_1, *wild-type* ♀ ♀ + F_1 *wild-type* ♂ ♂.

Apr. 3, 1913.	Wild-type.		Comma.		Pink.		Comma pink.	
	♀	♂	♀	♂	♀	♂	♀	♂
M 24.....	147	163	5	.....	55	67	3	.....

The F_2 from the cross of comma by vestigial gave 18 females and 1 male with comma, but not one of these was vestigial. While these numbers are not large enough to prove that comma is second-chromosome, the probablity is high that it is.

TABLE 76.—P_1, *comma* ♀ × *vestigial* ♂. F_1 *wild-type* ♀ ♀ + F_1 *wild-type* ♂ ♂.

Apr. 3, 1913.	Wild-type.		Comma.		Vestigial.		Comma vestigial.	
	♀	♂	♀	♂	♀	♂	♀	♂
M 23.....	282	262	18	1	68	73	0	0

Comma reappeared in the experiments involving "squat" and followed this second-chromosome mutant in distribution in such a way as to make it practically certain that comma is also in the second chromosome.

VALUATION OF COMMA.

Aside from carrying on a stock of commas for a few generations and noting that the percentage of commas was seldom above 30, nothing further was done with this mutant because of the poorness of the character. When present, the character comma was perfectly sharp and clear, even when, as was quite often the case, it showed on only one side of the thorax.

Perfectly definite and trustworthy classifications can be made with characters such as these, but they are very inefficient, since only flies actually showing the character can be considered. The conclusions drawn from such data are not so safe as the classification, since it has been our experience that such characters are particularly sensitive to intensification or repression (in percentage of appearance) by the other genes present in the crosses.

MORULA (*m*$_r$).

(Plate 10, figures 3*a* and 3*b*.)

ORIGIN OF MORULA.

In the F_2 from the cross of peach (third-chromosome recessive, allelomorph of pink) by wild, two red males were found that had only very inconspicuous bristles on the thorax (M 20, March 8, 1913).

INHERITANCE OF MORULA.

These two males were out-crossed to wild females, and in F_1 produced only wild-type males and females. Four F_1 mass-cultures were made up, and in F_2 these produced (table 77) a total of 1,950 flies, of which 432 or 22.2 per cent were "spineless." The "spineless" flies were exactly as numerous among the F_2 females as males, from which it follows that the character is an autosomal recessive mutant. There was found to be some little difficulty in classification, and it is certain that a few of the genetically spineless individuals were included among the wild-type; this was especially true in culture M 42.

TABLE 77.—*P_1, "spineless" (morula) ♂♂ × wild ♀♀; F_1 wild-type ♂♂ and ♀♀ inbred en masse.*

Apr. 3, 1913.	Wild-type ♀.	Wild-type ♂.	"Spineless" ♀.	"Spineless" ♂.
M 31.....	208	191	63	60
M 31r.....	164	176	44	43
M 42.....	126	122	26	34
M 43.....	264	262	83	79
Total....	767	751	216	216

FEMALE STERILITY OF MORULA.

The next step attempted was to secure a pure stock of "spineless" by mating together *en masse* several of the F_2 individuals from M 31.

It was discovered that this stock culture was not showing any larvæ, so the flies were put in a fresh bottle and several other "spineless" males and females added. This culture also failed. The F_2 cultures had been thrown away meanwhile, so that it was not possible to start other stock cultures. The flies were then taken from the mass-culture that had failed, separated as to sexes, and the males out-crossed to wild females and the females to wild males.

The out-cross of the males was successful, but the female out-cross failed entirely. The trouble, then, was confined to the females, which so far as tested (about 15 females) had been entirely sterile both with similar males and with wild males.

The "spineless" females that hatched in the F_2 from the male out-cross were tested very extensively. Probably as many as 150 such

females were tested either singly or in mass-cultures, and no offspring were produced. On the other hand, the males were entirely fertile. This case was the third to show female sterility. Rudimentary females usually gave no offspring, but occasionally gave a very few which were found to be almost entirely female. An extensive series of outcrosses of rudimentary females (carried out in 1912 by Bridges) produced a total of 623 females and only 9 males.

The sex-linked character "fused" was likewise found to be sterile as to its females and fertile as to its males, though no such extensive tests were made as in the case of rudimentary and "spineless." Since these three, several other mutations whose females were completely sterile and three mutations with completely sterile males have been found. All of these mutations with unisexual sterility have been turned over to Miss Clara J. Lynch for further investigation.

After the failure to maintain "spineless" stock by use of the females, the stock was run by mating in each generation "spineless" males by wild-type sisters heterozygous for the gene.

ARRANGEMENT OF THE FACETS OF MORULA.

At this time it was noticed that there was present in the "spineless" cultures a new type of eye modification, which was called "morula," since in it the facets of the eye had lost their regular pattern and were crowded together irregularly, much like the drupelets of a mulberry. The facets tended to "round up" and become more strongly convex, and to become more circular than hexagonal in outline (plate 10, fig. 3 *b*). The facets were also irregular in size and color, large ones being usually darker than those smaller. The eye as a whole was somewhat smaller than normal and more convex. The light reflected from the surface was broken up into many twinkling points by the tiny hairs which were found to be pointing in all directions instead of only radially (figs. 3 *b* and 3 *c*). This feature is common to several of the later discovered moruloid mutations and is the reason for the name "star" borne by the most useful of them all.

Most of the morula flies were found to be also "spineless;" several counts established the fact that every "spineless" was at the same time "morula," but that only about 90 per cent of the "morulas" were "spineless." This fact led to the suspicion that the two characters were effects of the same gene and that while the "morula" was a constant index of the mutation, the "spineless" might be called an "accessory" character. This agreed with the difficulty encountered previously in classifying "spineless" and with the too small proportion separated out. The differences between the numbers of "morula" and "spineless" flies gave a measure of the unreliability of "spineless." Throughout subsequent experiments attention was paid to the relation of "spineless" and "morula," and since no case was found in which a

"spineless" was not also "morula," the conclusion that they are effects of the same gene is justified. The name "morula" was then retained, and the name "spineless" was given to the third-chromosome recessive which now bears it.

The "spineless" flies must have had the "morula" character at the time of their discovery, and this character must have passed unnoticed for several generations, during which several hundred such flies were examined. That such a conspicuous character should be present and be overlooked would be almost unbelievable were it not that many other similar failures have been made, notably in the cases of dachs (see p. 216) and club. In the case of club, Morgan found and worked with the wing-character for many generations without discovering the absence of the pleural bristles which is the constant index of the mutant, Bridges surpassed this by finding in another stock this same mutant, which he worked with as a bristle character, and entirely failed to recognize the far more conspicuous wing modification which was present.

CHROMOSOME CARRYING MORULA.

To determine whether the gene for "morula" was in the second chromosome, a morula male was out-crossed to a curved female and three F_2 pair cultures raised (table 78).

TABLE 78.—P_1, *morula* ♂ × *curved* ♀; F_1 *wild-type* ♀ + F_1 *wild-type* ♂.

June 28, 1913.	Wild-type.	Curved.	Morula.	Curved morula.
M 48.....	55	22	26	0
M 49.....	56	22	24	0
M 50.....	97	42	54	0
Total.....	208	86	104	0

The F_2 ratio was plainly a 2 : 1 : 1 : 0 ratio, with no double recessives, which proved that morula was in the second chromosome.

An attempt to obtain the curved morula double recessive from F_3 or F_4 matings failed, but in F_4 from a similar cross of morula to black the double recessive was readily obtained.

LOCUS OF MORULA.

From this difference in the ease of obtaining the double recessive it was suspected that morula was nearer to curved than to black in the second chromosome.

It became apparent in F_3 that the double recessive black morula would be obtained in F_4, since some of the F_2 black females and morula males mated together had produced in F_3 a few black flies which were then known to be heterozygous for morula and which must produce black morula offspring among their progeny when inbred. Accordingly, by making an F_1 mating of morula male by black female at the

same time that the F_3 blacks were inbred, both the F_1 flies and the double recessive necessary to test them were obtained at the same time and a generation saved.

Three back-cross cultures were raised from pairs (table 79), which gave a total of 755 offspring, of which 353 or 46.8 per cent were cross-overs. This was very free crossing-over, comparable with the black arc and black balloon values previously found.

TABLE 79.—P_1, *black* ♀ ♀ × *morula* ♂ ♂; F_1 *wild-type* ♀ × *black morula* ♂ *of stock.*

Sept. 9, 1913.	Non-cross-overs.		Cross-overs.	
	Black.	Morula.	Black morula.	Wild-type.
II 93.....	66	89	73	62
II 95.....	59	49	51	55
II 96.....	73	66	59	53
Total....	198	204	183	170

TABLE 80.—P_1, *black arc* ♀ × *morula* ♂; *B.C.*, F_1 *wild-type* ♀ × *black arc morula* ♂ *from stock.*

Aug. 4, 1914.	$\frac{b\ a}{m_7}$		$\frac{b \mid m_7}{\mid a}$		$\frac{b\ a \mid m_7}{\mid}$		$\frac{b \mid}{\mid a \mid m_7}$		Total.
	Black arc.	Morula.	Black morula.	Arc.	Black arc morula.	Wild-type.	Black.	Arc morula.	
364.....	79	78	66	65	7	9	6	3	313
365.....	62	67	61	57	5	5	2	1	260
366.....	107	101	74	93	15	7	6	2	405
367.....	77	86	57	72	12	13	.....	5	322
368.....	65	69	45	59	4	10	5	4	261
369.....	74	77	47	63	7	9	3	4	284
383.....	126	105	88	81	12	10	5	3	430
384.....	71	80	65	66	3	9	4	2	300
385.....	66	73	58	52	4	6	3	6	268
386.....	45	52	31	41	3	3	3	1	179
387.....	112	63	74	64	7	14	3	2	339
Total.....	884	851	666	713	79	95	40	33	3,361

From a comparison of these values and from the fact that curved and morula had not crossed over readily, it was concluded that the locus of morula must be close to that of arc.

An effort was made to obtain triple-recessive black arc morula and black balloon morula. This latter stock was not obtained at all, and the former was secured only after repeated matings of (F_2, F_4, and F_6) black arc and black morula flies.

Triple back-crosses for the loci black, arc, and morula were made in pairs in each of the four possible ways in order to balance the inviability completely. The numbers are not equal in the different experi-

ments, which should be the case to secure the most perfect balancing of the inviability (tables 80, 81, 82, and 83).

The grand totals for these four complementary back-cross experiments furnished 6,794 flies, of which 3,469 were non-cross-overs, 2,791 cross-overs between black and arc, 374 cross-overs between arc and

TABLE 81.—*P_1, black arc morula ♂ × wild ♀; B.C., F_1 wild-type ♀ × black arc morula ♂ of stock.*

Aug. 19, 1914.	b a m_7		b | a m_7		b g | m_7		b | a | m_7		Total.
	Black arc morula.	Wild-type.	Black.	Arc morula.	Black arc.	Morula.	Black morula.	Arc.	
443.....	39	66	25	41	3	9	2	2	187
444.....	89	113	107	87	7	10	1	4	418
445.....	89	85	109	56	6	11	12	11	379
453.....	28	26	22	23	1	5	1	1	107
454.....	56	45	31	49	8	9	1	3	202
465.....	36	48	22	40	12	9	3	6	176
466.....	59	81	50	72	1	11	2	2	278
467.....	49	74	28	46	4	11	4	2	218
479.....	69	75	51	53	13	7	3	7	278
Total....	514	613	445	467	55	82	29	38	2,243

TABLE 82.—*P_1, black morula ♂ × arc ♀; B.C., F_1 wild-type ♀ × black arc morula ♂ of stock.*

Sept. 8, 1914.	b m_7 | a		b | a | m_7		b | a m_7		b | a | m_7		Total.
	Black morula.	Arc.	Black arc.	Morula.	Black.	Arc morula.	Black arc morula.	Wild-type.	
477.....	21	26	27	27	3	2	2	2	110
510.....	37	30	34	36	1	6	.....	.....	144
511.....	62	57	35	44	9	6	.....	1	214
Total.....	120	113	96	107	13	14	4	3	468

TABLE 83.—*P_1, black ♀ × arc morula ♂; B.C., F_1 wild-type ♀ × black arc morula ♂, from stock.*

Sept. 27, 1914.	b a m_7		b | a m_7		b m_7 | a		b | a | m_7		Total.
	Black.	Arc morula.	Black arc morula.	Wild-type.	Black morula.	Arc.	Black arc.	Morula.	
571.....	80	69	58	61	9	8	2	2	289
572.....	51	52	40	58	2	5	4	3	215
573.....	56	66	35	45	4	8	2	2	218
Total....	187	187	133	164	15	21	8	7	722

morula, and 160 were double cross-overs (table 84). The total cross-overs between arc and morula were 534 or 7.9 per cent. The black arc cross-over value was 43.4 per cent and the black morula 46.6 per cent, which agrees with the 46.8 per cent found in the former black morula back-cross. These values and the smallness of certain classes thereby known to be double cross-over classes established the position of morula to the right of arc, and 7.9 units distant.

TABLE 84.—*The four complementary back-cross experiments giving data on the relations of black arc, and morula.*

Aug. 4, 1914.	———	—|—	—||—	—|—|—	Total.	Cross-over values.		
						b a	*a* m_r	*b* m_r
$\frac{b\ a}{m_r}$	1,735	1,379	174	73	3,361	43.2	7.4	46.2
$\frac{b\ a\ m_r}{}$	1,127	912	137	67	2,243	43.6	9.1	46.7
$\frac{b\ m_r}{a}$	233	203	27	5	468	44.4	6.8	49.2
$\frac{b}{a\ m_r}$	374	297	36	15	722	43.2	7.1	4.61
Total.....	3,469	2,791	374	160	6,794	43.4	7.9	46.6

The coincidence of 69.1 per cent $\left(\frac{160\times6794\times100}{2951\times534}=69.1\right)$ is rather low for distances so long and indicates that a cross-over between arc and morula is only 69 per cent as likely to be accompanied by a cross-over between black and arc, as would be the case if the two regions were independent.

TABLE 85.—*Summary of the cross-over data involving morula.*

Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Streak morula.	876	405	46.3	Aug. 24, 1913	Bridges	S_k; $\frac{S_k}{m_r}$ B. C.; II 64–II 97.
Black morula..	755	353	46.8	Sept. 28, 1913	Do.	m_r; $\frac{b}{m_r}$ B. C.; II 93–II 96.
	6,794	3,165	46.6	Aug. 4, 1914	Do.	m_r; *ba mr* balanced B. C.; 364–573.
	7,549	3,518	46.6			
Arc morula....	6,794	634	7.9	Aug. 4, 1914	Do.	m_r; *ba mr* balanced B. C.; 364–573.

The purple arc speck back-cross (table 54) had given 5.9 units to the right of speck. Morula is therefore situated about 2 units to the right of speck. A summary of all the cross-over data including morula is given in table 85, from which the locus of morula is calculated as 106.3 on the basis of star as the zero-point.

VALUATION OF MORULA.

The locus of morula is the farthest to the right of the workable mutants, and for this reason is valuable, though speck, which is less than 2 units to the left of morula, will probably be used in the majority of cases. There is another reason for the preference of speck over morula in general—that morula would interfere in classification with star, which is the most useful of the second-chromosome mutants, while speck interferes neither with star nor any other second-chromosome mutant. The female sterility of morula also limits its usefulness in complex experiments. On the other hand, the character morula is exceptionally easy and certain in its separability from the wild-type, being surpassed in this regard by no other second-chromosome mutant except vestigial. Its viability also is excellent.

APTEROUS (a_p).

(Plate 7, figure 5.)

ORIGIN OF APTEROUS.

The mutant called "apterous" was first found by Miss Wallace in the white miniature stock in August 1913. This form continued to appear as occasional individuals, both males and females, for some months, from which it was concluded that the mutant was an autosomal recessive for which only a few white miniature flies were heterozygous. Several attempts to breed apterous individuals to one another or to other flies failed, and it was thought that the form was completely sterile.

DESCRIPTION OF APTEROUS.

The most striking feature of this mutant is, as its name implies, the total absence of wings, the vestiges being in most cases a mere roughness. The balancers also are reduced in the same manner as the wings, a condition that was first found in the case of vestigial. The apterous flies are small in size, rather pale in color, and markedly sluggish in movement; they easily become entangled in food or cotton and drown or dry up. Even when kept very carefully under the best conditions they seldom live more than three or four days.

INHERITANCE OF APTEROUS.

At this stage Metz (C. W. Metz, Am. Nat. 1914, pp. 675–692) began work with the mutation and found that the failure to breed was largely because the males were too weak to copulate and the females produced few or only rudimentary eggs. In only three cases out of more than a hundred did apterous females give offspring when crossed to normal males, and there was correspondingly only a single case of fertilization by an apterous male. From these crosses and from inbreeding other pairs heterozygous for apterous there were produced a total of 1,405 wild-type to 450 apterous flies, or 24.3 per cent apterous, which is a remarkably close approach to expectation, in spite of the weakness of the apterous flies.

CHROMOSOME CARRYING APTEROUS.

Metz crossed an apterous male to a vermilion female and, as expected, the apterous character and vermilion showed no linkage in F_2. The total offspring from pair matings in this line was 1,498 wild-type to 369 apterous, or 19.8 per cent, which is a poorer viability than before, but not as low as in the sister culture raised *en masse*. The mass-cultures gave only 699 apterous among 4,539 offspring, and the low percentage (15.4) is the result of the crowding that always occurs in mass-cultures.

An apterous female was successfully crossed to a pink male and there were produced in F_2 402 wild-type, 114 apterous, 111 pink, and 34 apterous pink flies, which is an approach to a 9:3:3:1 ratio and proved that apterous was not third-chromosome.

No successful mating of apterous by black was made, so that flies heterozygous for apterous had to be used in the P_1 instead. From F_1 pairs, heterozygous for apterous in both parents, there were produced 414 wild-type, 136 apterous, 155 black, and 0 black apterous flies. This 2:1:1:0 ratio demonstrated that apterous is in the second chromosome.

LOCUS OF APTEROUS.

In determining the locus of apterous, Metz took advantage of the fact that flies heterozygous for black are darker than the wild-type, that is, he classified black as a dominant as well as a recessive character in F_2. In the above F_2 from the cross of apterous (heterozygous) to black, Metz observed no apterous flies that seemed to be heterozygous for black and correspondingly no long-winged flies that were not heterozygous for black. While this classification can not be accurate, it is nevertheless certain that if very many such cross-over flies had been present this fact could have been noted. The locus of apterous is therefore not far from that of black in the second chromosome.

APTEROUS BY REMUTATION.

In working with the eosin modifier "cream c" Bridges found a mutant character which in appreance agreed at every point with the previously known apterous (cultures 5588, 5631, October 16, 1916). This experiment, in which apterous reappearance had come from the P_1 mating of the original cream c female (found in eosin stock) and a star-dichæte male (dichæte is a third-chromosome dominant). The F_1 mating had been of (eosin) star-dichæte sons and wild-type daughters in pairs. The apterous character appeared in 63 individuals (males and females equally numerous) in a total of 449 in two separate cultures (5588 and 5631). The percentage of apterous was thus only 14.0, which indicates a rather poorer viability than was shown in the culture of Metz.

CHROMOSOME CARRYING APTEROUS.

The apterous character was distributed at random with respect to both eosin (first chromosome) and dichæte (third chromosome). Not one of the star F_2 individuals was apterous. In fact, the F_2 ratio of 258 star : 128 wild-type : 63 apterous : 0 star apterous approached the 2 : 1 : 1 : 0 ratio expected if apterous is in the second chromosome (table 86). There was observed a very free crossing-over between

TABLE 86.—*P_1, cream c ♀ (heterozygous for apterous) × star dichæte ♂; F_1 wild-type ♀ + F_1 star dichæte ♂.*

Oct. 20, 1916.	Star.	Wild-type.	Apterous.	Star apterous.
5588.....	104	67	37	0
5631.....	154	61	26	0
Total.....	258	128	63	0

cream c and apterous, and this fact, in connection with the star apterous result, proved that the apterous mutation had been present in the cream c female, which was presumably heterozygous for it. That this appearance of apterous is due to a second and independent occurrence of the act of mutation in the apterous locus can not be doubted.

TABLE 87.—*F_2, F_3 and F_4 star ♀ $\left(\frac{S'+}{+\ a_p}\right)$ + F_2, F_3, and F_4 wild-type ♂ $\left(\frac{+}{a_p}\right)$.*

Nov. 18, 1916.	Star.	Wild-type.	Apterous.	Star apterous.
5834.....	95	95	24	9
6014.....	3	5	.....	1
6070.....	57	60	6	3
Total.....	155	160	30	13

TABLE 88.—*F_4 star ♀ $\left(\frac{S'+}{+\ a_p}\right)$ + F_4 star ♂ $\left(\frac{S'+}{+\ a_p}\right)$.*

Jan. 1, 1917.	Star.	Wild-type.	Apterous.	Star apterous.
6347.....	24	6	7	9

LOCUS OF APTEROUS.

An opportuinty for determining the locus of apterous was provided by the above material. All attempts to back-cross the F_2 star females, a majority of which were heterozygous for apterous,

$$\left(\frac{S'\ +}{+\ a_p}\right)$$

by apterous males failed as expected, because of the sterility of the apterous males. However, a few matings between such females and

wild-type brothers heterozygous for apterous succeeded (table 87). There were produced a total of 358 flies, of which only 43 or 12.0 per cent were apterous. Of these 43 the star apterous cross-overs numbered 13 and the simple apterous non-cross-overs 30.

These flies are comparable in viability and the percentage of crossing over can be calculated directly from them as 30.2. Another calculation can be made from the not-apterous flies, of which there were 155 stars and 160 wild-type. Both these classes are composite, the stars comprising two non-cross-over and one cross-over class ($2n+x=155$), while the wild-type flies comprise one non-cross-over and two cross-over classes ($n+2x=160$). From these equations $n=50$ $x=55$, or the percentage of crossing over is 52.3.

One cross of star female by star male both $\left(\frac{S' \times}{\times a_p}\right)$ produced offspring only one of whose classes is composite (table 88). As before, the star apterous flies are cross-overs ($x=9$) and the apterous non-cross-overs ($n=7$) which gives 9 cross-overs out of 16 or 56 per cent. The wild-type class is a simple non-cross-over ($n=6$) to be compared with the complex class star ($2n+x=24$). From these equations $x=6$ and $n=9$, or the percentage of crossing over is 40.0. The total amount of crossing-over data derived above gives the equivalent of 169 flies, of which 83 or 49.0 per cent are cross-overs. From a comparison of this value (49.0) with the cross-over values given by star and other second-chromosome genes, the locus of apterous is found to be most probably somewhat to the right of black. This locus agrees perfectly with the position found by Metz on the basis of the black-apterous F_2 described above. A position at 48.5 is indicated as approximately correct.

C_{II_l} AND C_{II_r}.

A paper by Sturtevant, giving a full account, with summaries, of the work done on the two second-chromosome cross-over variations C_{II_l} and C_{II_r} appears herewith (Part III), to which the reader is referred.

CREAM II (Cr_{II}).[1]

(Plate 5, figure 11.)

ORIGIN AND DESCRIPTION OF CREAM II.

A pure stock of the sex-linked eye-color eosin shows a strong sexual bicolorism; that is, the eye-color of the eosin female is a rather deep pink only slightly yellowish, while the eye-color of the eosin male is a pinkish yellow much lighter in tone than the color of the female (see Morgan, 1912, for an account of the origin of eosin and a colored plate showing this difference in color). Eosin females and males maintain

[1] A short reference to the case of cream II, and a discussion of its bearing on the question of multiple factors was made in the "Mechanism of Mendelian Heredity," p. 203.

this constant difference in color wherever they reappear after crossing, and all double recessives involving eosin, for example, eosin vermilion and eosin pink, are likewise bi-colored (Morgan and Bridges, 1913).

In carrying on a stock of non-disjunction by means of eosin, Bridges found (July 13, 1913) that certain flies were showing an eye-color considerably paler than the standard eosin. Investigation of these "cream"-colored flies showed that they were double recessives, being eosin plus a specific modifer of eosin eye-color. Thus, a pure stock of the modifier would look precisely like a stock of wild flies.

Shortly after the discovery of the first cream (cream a), a second cream appeared (September 15, 1913) among the eosin males and females of a stock culture of lethal 2.[1] Wild-type females heterozygous for lethal 2 had been crossed to eosin miniature males, and the F_1 wild-type daughters again crossed to eosin miniature males. The mothers of the culture which gave the creams were therefore wild-type females heterozygous for eosin and miniature as well as lethal 2 $\left(\frac{w^e + m}{+ l_2 +}\right)$, while the fathers were eosin miniature. The cream males and females which appeared were much paler than cream a, though like cream a they were a light, translucent yellow with little or no pinkish tinge. None of the not-eosin flies were different in color from normal red flies.

A careful examination of the stock of eosin miniature failed to show any flies that did not have the standard eosin eye-color, and no lighter eye-color has ever subsequently shown itself in this stock. It is evident that the gene for the modification had been present in the wild-type flies of the lethal 2 stock, but had been unsuspected so long as eosin was not present as a base. The demonstration that the cause of the observed dilution of eosin was a gene behaving in inheritance like the other mutant genes was easily made.

INHERITANCE OF CREAM II.

One of these cream males was out-crossed to a wild female. Among the F_2 flies the creams reappeared, and, as in the parallel case of cream a, the not-eosin flies were all indistinguishable from one another and from wild flies in color. The F_2 result resembles that obtained with cream a, except that, as stated, the new cream was considerably paler; and it was further discovered that besides the creams, approximately 50 per cent of the eosin males were intermediates between eosin and this cream, that is, cream II diluted eosin even in heterozygous form, so that the eosin sons were visibly as well as genetically in the ratio 1 eosin : 2 eosin heterozygous for cream II : 1 eosin pure for cream II. The entire ratio, disregarding sex, approximated 12:1:2:1, the 12 being the red-eyed flies.

[1] This culture was part of a generation which succeeded generation Q, table 22, p. 111, Morgan, 1914, and which gave results similar to the results of generations J to Q of table 22.

STOCK OF CREAM II.

From the F_2 a few cream males were selected and bred to their sisters, all of which were wild-type in appearance, though a quarter of them were homozygous for the cream gene (not-eosin creams). This mass-culture gave the expected cream females and males, from which a pure-breeding stock was made up. There was a difference in the color of the males and females of this pure stock, the difference being of the same order as the normal bicolorism of eosin.

A complete separation of the eosin from the eosin heterozygous for cream had not been attempted in the original F_2 culture. In order to observe the heterozygous condition more closely a cream male from the pure stock was out-crossed to an eosin female. The F_1 flies both males and females (culture M 68, intermediate males 73, intermediate females 88)[1] were lighter in eye-color than standard eosin, though the difference between eosin and these heterozygotes was less than the difference between the heterozygotes and the pure cream.

TABLE 89.—*F_2 offspring from the cross of a cream male to an eosin female.*

Dec. 6, 1913.	Females.			Males.		
	Eosin.	Hetero-zygous cream.	Cream.	Eosin.	Hetero-zygous cream.	Cream.
M 77.....	30	57	29	29	46	25
M 78.....	19	49	16	14	30	15
M 79.....	23	43	19	18	34	20
M 95.....	14	32	11	13	27	13
Total.....	86	181	75	74	137	73

Among these F_2 offspring (table 89) there were six different eye-colors; among the males, the same three that occurred in the original F_2, and among the females three colors which, though corresponding genetically to the classes among the males, were darker in eye-color. The cream female is lighter than the eosin male, while the heterozygous cream female is somewhat darker than the eosin male. In order from the darkest (a deep slightly yellowish pink) to the lightest (a pale translucent yellow) the six colors are: eosin female, heterozygous cream female, eosin male, heterozygous cream male, cream female, cream male. The females were first separated from the males. Then

[1] One of the 88 intermediate daughters had only three segments to her abdomen instead of the usual five. This female (figured by Morgan, 1915, p. 425, text-figure 3*a*) was the founder of a new type of abnormal segmentation of the abdomen—"patched." The segments were reduced in number (as in the first specimen), or, more typically, were cut sharply into oblique or triangular pieces which were patched together as illustrated in figures *b* to *f*, of plate 11. This character was recessive, but it generally reappeared in very much less than a quarter of the F_2 offspring. The usual causes for such deficiencies are poor viability, partial or complete dependence for realization on the coaction of one or more other genes, or failure to be developed in all the flies fluctuations of pure for the gene, whether from environmental differences or because the normal genetically the character overlap the wild-type. The gene for patched was in the second chromosome, as shown by its strong linkage to the cream.

in each sex the pure creams were separated from the others, and finally the more difficult separation of heterozygous cream from eosin was undertaken. The separation of the creams from the other colors is accurate, but the final separation, that of the heterozygous creams from the eosins, must be regarded as only a close approximation. The sharp 1:2:1 ratio (160:318:148) which was obtained from this separation probably represents among the eosins a small number of the darkest heterozygotes, while the lightest of the pure eosins were likewise classified among the heterozygotes. Probably 10 per cent of the combined eosin and heterozygous cream class overlapped enough so that the separations might or might not correspond to genetic differences. One test of the correctness of the classification of intermediates was made. From culture M 79 a heterozygous male and a heterozygous female were selected, and the results (culture M 75) showed that both individuals were of the supposed type.

No attempt has been made to secure a stock homozygous for the cream gene but without eosin. The cultures and experiments in which such not-eosin creams must have constituted one-fourth of the wild-type flies prove that such a stock could not be distinguished by inspection from a wild stock.

That the action of cream II is specific to eosin was suggested by crosses of cream with vermilion (X chromosome) and with pink (third chromosome). A careful examination of the F_2 flies from these crosses showed no dilution of either vermilion or pink by the cream, that is, the double recessives vermilion cream and pink cream (not-eosin) are indistinguishable from vermilion and pink respectively.

LINKAGE METHOD OF ANALYSIS FOR MULTIPLE-GENE CASES.

The proper method of study for cases of multiple factors or of modifiers is by means of linkage experiments, whereby all guesswork as to the number and effect of modifiers can be eliminated. In *Drosophila* such a study is rendered particularly easy by the fact that in the male there is no crossing-over of any of the chromosomes. In consequence, if two recessive genes which belong to the same chromosome, *e. g.*, black and vestigial of the second chromosome, enter the cross from opposite parents ("repulsion"), the F_2 never shows flies which have both these mutants at the same time. The double recessive class is entirely unrepresented, and the 2:1:1:0 ratio of "absolute repulsion" results. This ratio holds, whatever may be the amount of crossing-over in the female, for the lack of double-recessive sperm prevents the double-recessive eggs from revealing themselves. This ratio is in marked contrast to the 9:3:3:1 ratio, which obtains when the two genes belong to different chromosomes, *e. g.*, curved of the second chromosome and ebony of the third chomosome.

The light color cream was known to be eosin plus a recessive modifier which belonged to an autosome group. To find whether this group

a
b
c
d
e
f

was that of the second chromosome, a cream male (from pure stock) was out-crossed to a curved female, curved being a recessive wing-character whose gene is known to belong to the second chromosome (Bridges and Sturtevant, 1914). A pair of F_1 wild-type flies inbred gave the results of table 90.

TABLE 90.—*F_2 from the cross of cream II male to curved female.*

Feb. 20, 1914.	Not-eosin (♂ + ♀).		Eosin (males only).			
	Wild-type.	Curved.	Eosin.	Eosin curved.	Cream II.	Cream II curved.
70.....	155	64	37	14	15	0

Since cream only shows itself where eosin is already present, we may disregard all the flies of culture 70 except those with eosin eyes. These eosin flies are obviously in the ratio 2:1:1:0 which is expected if the cream gene is in the second chromosome, through the flies are too few to prove the point.

TABLE 91.—*P_1, cream II ♂ × eosin black ♀. F_1 heterozygous cream ♀ × F_1 heterozygous cream ♂.*

F_1 cultures. Mar. 16, 1914.	Heterozygous cream ♀♀.				Heterozygous cream ♂♂.			
119.....	51				41			
329.....	15				18			
Total....	66				59			
F_2 cultures. Aug. 3, 1914.	Eosin.		Eosin black.		Cream II.		Black cream II.	
	♀	♂	♀	♂	♀	♂	♀	♂
372.....	50	38	24	15	14	25	0	0
398.....	57	42	18	31	19	19	0	0
399.....	69	79	36	43	43	39	0	0
400.....	48	59	33	17	24	24	0	0
Total....	224	218	111	106	100	107	0	0
	442		217		207		0	

A more efficient experiment than this last was carried out by making all the flies of the experiment eosin, in which case the 2:1:1:0 ratio involved all the offspring rather than only a quarter, as in culture 70. A stock of eosin black was made up (black being a second-chromosome mutant) and a female of this stock was outcrossed to a cream II male. The F_1 and F_2 results are given in table 91. All of the F_1 flies and half the F_2 flies were of the intermediate color of the heterozygous cream. In the F_2 these intermediates were classified along with the pure eosins, so that the cream was treated as though a strict recessive.

The F_2 ratio of 442 : 217 : 207 : 0 is a very close approximation to a 2 : 1 : 1 : 0 ratio and proves that the gene for cream is in the second chromosome (cream II).

A similar experiment in which cream was crossed to eosin-ebony (ebony being a third-chromosome mutant, see Sturtevant, 1914) gave a typical 9 : 3 : 3 : 1 ratio (table 92), which agrees with the fact that the cream gene is not in the third chromosome.

TABLE 92.—*F_2 from the cross of cream II ♂ by eosin ebony ♀.*

Mar. 31, 1914.	Eosin.	Cream II.	Eosin ebony.	Cream II ebony.
154.....	61	21	18	4
161.....	85	28	35	14
162.....	134	37	36	10
Total...	280	86	89	28

In order to find the locus of cream within the second chromosome it would have been necessary to run two linkage experiments in which all the flies were eosin; thus, one of these might have been cream II by eosin black and a back-cross of the F_1 female to black cream males, and the other a similar back-cross in which curved was used in place of black. The amount of crossing-over between black and curved was known to be about 27 per cent. The two values black cream and curved cream which would be found by two such experiments (both values might, of course, be found from a single suitably devised experiment) would enable the locus of cream to be calculated with considerable accuracy. While much is to be learned of the mechanism of crossing-over from a study of the relative distributions of loci within various regions of the chromosome, yet in the case of cream II it was thought that the compensation would not be worth the effort. Any further use of cream II in other linkage experiments would involve the "eosinization" of all the stocks used. In the case of certain of the later creams, an approximate location of the gene within the chromosome has been made, but such location was made easier by the discovery of certain dominant mutations which were not available at the time the work on cream II was finished.

TREFOIL (*tf*).

(Plate 5, fig. 6; plate 8, fig. 1.)

ORIGIN AND STOCK OF TREFOIL.

The character trefoil was found by Morgan about November 1913, and a pure-breeding stock was secured without difficulty.

DESCRIPTION OF TREFOIL.

The character trefoil is quite variable in the intensity of pigmentation, as is the case with all of the thorax pattern characters. The

distribution of the pigment is very definite, however. The scutellum is largely or entirely dark and the base of the trident pattern on the thorax is broader and much heavier, while the prongs are scarcely darkened at all. The characteristic feature of trefoil is the presence of extra basal sections to the trident outside the regular region. These side areas are fully as dark as the central parts and extend forward even farther. Another region that is dark in trefoil but not in the other thorax patterns is a patch behind each eye on the back of the head. These eye-patches and the side-prongs are the main characters used in classifying trefoil.

INHERITANCE OF TREFOIL.

In out-crosses to wild, trefoil behaved as an autosomal recessive, giving only wild-type flies in F_1, and reappearing as about a quarter of the F_2 flies.

CHROMOSOME CARRYING TREFOIL.

F_2 from the cross of trefoil to pink gave a 9 : 3 : 3 : 1 ratio, while the corresponding cross to curved gave a 2 : 1 : 1 : 0 ratio, from which it was seen that the locus of trefoil is in the second chromosome.

LOCUS OF TREFOIL.

It was found that trefoil and black together gave a very dark fly which was distinguishable from black. With some difficulty a triple-recessive black strap trefoil stock was made up to test the locus of trefoil. This stock was never used, but from the indications met with in its synthesis it seemed probable that the locus of trefoil is not far from that of black, but between black and strap. This was confirmed roughly by a star trefoil back-cross which gave 42.1 per cent of crossing-over (S 34, tf 55, S tf 42, +23). The locus is thus at about 50.0 with reference to star.

VALUATION OF TREFOIL.

Considerable difficulty was met with in the classification of trefoil from the variability of the character, and for this reason there was no strong incentive to establish its locus or to use it in any way.

CREAM b (c_{rb}).

(Plate 5, fig. 11.)

ORIGIN OF CREAM b.

An eosin female from a stock of non-disjunction, when mated to a bar male, gave (culture 82, March 10, 1914) among the eosin sons one whose eye-color was as light as that of cream II or cream III. This male was out-crossed to a wild female and in F_2 gave creams among the eosin sons but no disturbance of the color of the not-eosin flies (cultures 183, 184, 185). The F_2 ratio was again 12 : 3 : 1, as in similar

crosses with other recessive specific dilutors. But the creams (cream b) which occurred in this F_2 were not as pale as any of the preceding creams.

From the circumstances of the appearance of cream b, viz, that it was observed in the F_1 of an out-cross and that as a single individual, we should expect it to be a dominant, but as a matter of fact it proved to be a recessive. It seems probable, in explanation, that more creams were actually present in this F_1 but were overlooked, since attention was distracted by the simultaneous appearance in the same culture of still another mutation (lethal 4), and more especially since the effect of cream b is rather slight. Only occasionally was one of the F_2 creams so marked as the grandfather, and the mutation might not have been recognized at all were it not that an extreme fluctuant had attracted attention. Since cream b is recessive, we must suppose that the gene was present in both parent stocks. It could have been present in the bar stock and been undetected because of the lack of eosin, without which it has no visible effect; and the character might readily have been present in the eosin non-disjunction stock and have been passed over as an age variation, since, as we ordinarily see flies from a stock culture, they are of all ages and of all corresponding densities of pigmentation.

CHROMOSOME CARRYING CREAM b.

A pure-breeding stock was made up for use in back-crossing. By this time we were in possession of a good second-chromosome dominant "star" and likewise of a perfect third-chromosome dominant

TABLE 93.—*B. C. offspring from the P_1 mating of an eosin star dichæte male to a cream b female and the back-crossing of the F_1 eosin star dichæte male to cream b females.*

Sept. 8, 1916.	Non-cross-overs.				Cross-overs (in male).			
	Eosin star.	Eosin star dichæte.	Cream b.	Cream b dichæte.	Star cream b	Star cream b dichæte.	Eosin.	Eosin dichæte.
5155 ♀	20	25	26	12	0	0	0	0
5155 ♂	19	21	21	26	0	0	0	0
5409 ♀	14	21	18	26	0	0	0	0
5409 ♂	14	15	17	24	0	0	0	0
Total	67	82	82	77	0	0	0	0

"dichæte," which mutants have now become the most important in their respective chromosomes. By aid of these two dominants it is very easy to determine in a single experiment whether a given mutant is in the second or third chromosome. Thus, in the case of cream b, a stock of eosin star dichæte was made up and used in making a P_1 cross to the cream. Then F_1 eosin *males* which showed both star and dichæte and which were heterozygous for the recessive cream were back-crossed

to cream b females of stock. There is no crossing-over in the male of *Drosophila*, so that if cream b were in the second chromosome not one of the B. C. star offspring should be cream, while half of the dichæte should be cream and half not. If, on the other hand, the cream were in the third chromosome, then none of the B. C. dichætes should be cream, while the star and cream should assort at random. The experiment proved that the gene for cream b is in the second chromosome (table 93).

LOCUS OF CREAM b.

An (eosin) star female and a cream b male selected from the B. C. offspring gave in the next generation the amount of crossing-over between star and cream b (table 94). This value of 22.1 includes some double crossing-over, and the corrected or "map" distance is probably about 22.5. The chances are in favor of the cream b locus being to the right of star, since star happens to occupy the leftmost of the known loci.

TABLE 94.—*B. C. offspring given in F_2 by an eosin star female and a cream b male from table 93.*

Oct. 20, 1916.		Non-cross-overs.		Cross-overs (in female).	
		Eosin star.	Cream b.	Star cream b.	Eosin.
5593	♀	36	41	8	8
	♂	22	41	12	12
6532	♀	47	39	13	7
	♂	28	49	11	15
Total....		133	179	44	42

PINKISH.

(Plate 5, fig. 12.)

ORIGIN OF PINKISH.

In the fall of of 1913 a stock of eosin black had been made up with which to test the chromosome group of cream II. In the following summer (July 27, 1914) Bridges noticed that a few of the males were somewhat lighter in eye-color than the others, but seemed chiefly noticeable because of the weakness of the yellow component of the eosin eye-color. The color of the regular eosin male is a pinkish yellow; the color of cream a, II, III, and b is nearly a pure yellow with little of the pinkish tinge, while this new color was somewhat the converse of this and was a pale pink with relatively little yellow.

One of these males mated to a sister gave all of the sons of this pinkish color and all the daughters of a similar color, which is, however, much harder to distinguish from standard eosin. It seems that this character is somewhat sex-limited in the same direction as eosin.

Pure stock of the mutation had been obtained at once through the happy selection of a pure pinkish female which had been taken to be simply an eosin female of somewhat lighter eye-color because of being freshly hatched.

CHROMOSOME CARRYING PINKISH.

Since pinkish appeared in a stock of eosin black, material was on hand to test the chromosome group at once. Accordingly, black pinkish females were out-crossed to eosin males and the F_2 eosin females, standard eosin in color, were back-crossed to black pinkish males. In the back-cross cultures half of the flies were not-black, and the not-black pinkish flies were seen to be less markedly "pinkish" in eye-color than the blacks. In the absence of black the eye-color was more nearly like that of the other creams, though the amount of dilution is less than in any of the other creams. In the first two of

TABLE 95.—*Offspring given by the F_1 eosin-eyed daughters from the out-cross of black pinkish females to eosin males, when back-crossed to black pinkish males.*

Sept. 13, 1914.	Non-cross-overs.		Cross-overs.	
	Black pinkish.	(Eosin).	(Eosin) black.	Pinkish.
525.....	70	81	71	24
526.....	36	29	32	22
2424.....	25	21	24	35
2425.....	24	27	29	31
2426.....	28	24	14	29
Total....	183	182	170	201

these back-cross cultures (table 95) males and females were classified together. Some question having been raised in regard to the accuracy of the separation of pinkish from eosin among females, the cross was repeated and the readily classifiable males (last three cultures) gave the same result as before. It was seen that the new or cross-over combinations were as numerous (51.4 per cent) as the original classes, and this independent inheritance was taken to mean that the gene for pinkish is not in the second chromosome. While this was a mistaken notion—the true relation being that the gene is so far away from black that in the female there is entirely free crossing-over—yet it led to the device of the efficient "double-mating" method of ridding a given stock of an undesired recessive.

THE DOUBLE-MATING METHOD.

If pinkish were in the third chromosome, then the presence of the black in the pinkish stock could be of no advantage, and might be a very serious handicap, since it would prevent the use of all our third-chromosome stocks containing ebony or sooty. The first step in the

elimination of black was to mate together some of the not-black pinkish flies of table 95. One-third of the not-black offspring of such pairs should be of the desired kind—that is, entirely free from black. Our task was then to pick out from the mixture of pure grays and grays heterozygous for black some pure gray males. In this special case we were aided by the fact that black happens to be slightly dominant—that is, the heterozygous blacks are somewhat darker than the pure grays. While this difference is not marked enough to be used regularly in classification, it enables us to pick out by inspection a greater proportion of pure grays than we would get by random selection. Four such males were selected as being probably free from black and were mated to eosin females. Into the same bottle with each pair of these flies was put a virgin (red-eyed) black female. The offspring from these two mothers are easily distinguished, since they are eosin-eyed if from the eosin mother and red-eyed if from the black mother. The offspring from the black mother constitute a test of whether the father were free from black, for in this case none of the red-eyed offspring hatching in the double-mating culture should be black, while if the father were heterozygous for black half of the red-eyed offspring should be black. Only one of the four cultures gave black offspring, and this culture was then discarded. . . . The eosin-eyed flies of the other three cultures were all heterozygous for pinkish, and at the same time free from black. By mating together some of these eosin-eyed flies pure pinkish offspring should be obtained as a quarter of the offspring. A more efficient method, and the one actually followed, was to save the fathers and mate them to their eosin-eyed daughters, since in this case half, rather than a quarter, of the progeny should be pure pinkish.

TABLE 96.—*B. C. offspring given by the F_1 eosin star dichæte sons, from the out-cross of a pinkish female to a star dichæte male, when back-crossed to pinkish females.*

Aug. 25, 1916.	Non-cross-overs.				Cross-overs (in the male).			
	(Eosin) star.	(Eosin) star dichæte.	Pinkish.	Pinkish dichæte.	Star pinkish.	Star pinkish dichæte.	(Eosin).	(Eosin) dichæte.
5029 ♀	10	12	10	9	0	0	0	0
5029 ♂	17	13	16	22	0	0	0	0
5266 ♀	22	20	18	21	0	0	0	0
5266 ♂	20	20	21	26	0	0	0	0
Total	69	65	65	78	0	0	0	0

In order to show by an actual test that the gene for pinkish is in the third chromosome, it was decided to take advantage of the fact of no crossing-over in the male and to run a back-cross test of a male heterozygous for pinkish and for the dominant third-chromosome character

dichæte. It was now realized that the back-cross tests of females heterozygous for pinkish and black had not excluded the possibility of pinkish being in the second chromosome, though they had shown that, if so, it could be only in one or the other end-region and not near black. Accordingly, exactly the same procedure was followed as in the tests for the location of cream b, that is, a pinkish female was out-crossed to a male which had the second-chromosome dominant star as well as dichæte. The F_1 eosin star dichæte males were then back-crossed to pinkish females. The result showed (table 96) that the gene for pinkish is in the second and not the third chromosome; for, as well as could be judged, none of the star flies were pinkish, while all the not-stars seemed to be pinkish, and also dichæte was present in half of both the star and the pinkish classes.

TABLE 97.—*B. C. offspring given by a star female from table 96, when back-crossed to a pinkish male.*

Sept. 23, 1916.	Non-cross-overs.		Cross-overs.	
	(Eosin) star.	Pinkish.	Star pinkish.	(Eosin.)
5267 ♀	19	30	19	20
5267 ♂	26	26	19	16
Total....	45	56	38	36

TABLE 98.—*F_2 offspring given by the F_1 wild-type females and F_1 eosin males, from out-cross of pinkish females to wild males.*

Oct. 28, 1916.	Wild-type.	(Not-eosin) pinkish?	Eosin.	Pinkish.
5678.....	121	5	76	24
5680.....	52	2	46	17
5703.....	63	14	59	18
5704.....	57	6	52	26
5705.....	80	3	67	18
Total....	373	30	300	103

LOCUS OF PINKISH.

In the light of this test, and from the fact that there was about 50 per cent of crossing-over between black and pinkish, we could place pinkish in either the extreme left or the extreme right end-region of the second chromosome. Fortunately, one advantage of the test just described is that it left us in possession of females heterozygous for star and for pinkish, and a back-cross test showed (table 97) that there is very free crossing-over between star and pinkish. Pinkish is known, therefore, to be in the right-hand end of the second chromosome, in the neighborhood of arc, speck, balloon, etc. Had the test given almost no crossing-over between star and pinkish, we should

have known that the gene for pinkish was in the left end, but this was not the case.

A test as to whether the pinkish gene would have any visible effect in the absence of eosin showed (table 98) that in a very small per centage of the flies homozygous for pinkish there is a very slight dilution. This dilution is, however, so slight that rarely could one be sure that the effect observed is due to dilution rather than to the slight normal fluctuation of the red.

PLEXUS (p_z).

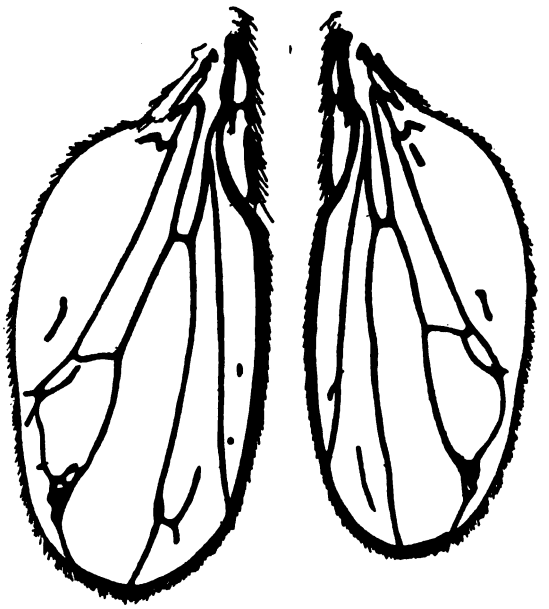
(Text-fig. 80.)

ORIGIN OF PLEXUS.

The venation character "plexus" was found by Bridges in a stock culture of the third-chromosome recessive spread wings (August 20, 1914, culture 557). Fully 10 per cent of the spread flies showed the plexus venation.

DESCRIPTION OF PLEXUS.

The most striking feature of plexus is a rather tangled knot of extra veins near the distal end of the fifth longitudinal vein and the posterior cross-vein, and another such knot near the distal end of the fourth longitudinal vein, with an extra vein running near the margins of the wing and connecting the two (see text-figure 80). Several other small sections of extra vein are often present in various parts of the wing, most of them lying free in the cells, but some being branches of or connected to the regular veins. These veins are all sharp and clear, without indefiniteness and discoloration such as characterize the extra veins of balloon. There is a very characteristic bend forward in the fourth longitudinal vein before it reaches the marginal vein.



TEXT-FIGURE 80.—Plexus venation.

In less extreme individuals the connecting vein may be quite absent and the knots much reduced. The branch from the posterior cross-vein running parallel to the fifth longitudinal vein and also the bend in the fourth vein are the most persistent of all the characters.

INHERITANCE AND CHROMOSOME OF PLEXUS.

One of the plexus spread males was out-crossed to a black female and produced in F_1 only wild-type flies, showing that the character was recessive. Several F_2 cultures were raised from pairs of the F_1 flies, and in these plexus reappeared as about a quarter of the F_2 individuals. The plexus venation was present equally among the F_2 females and males, from which it was known not to be sex-linked. Likewise plexus was known not to be third-chromosome from the free recombination of plexus and spread among the F_2 individuals. This distribution of plexus and spread might have been thought due to very free crossing-over between spread and plexus instead of the random assortment, were it not that the black and plexus appeared in the typical 2:1:1:0 ratio, which showed that the locus of plexus is in the second chromosome.

From the F_2 cultures black and plexus flies were crossed to each other, with the two-fold purpose of obtaining the double-recessive form and of elimination the third-chromosome recessive spread.

LOCUS OF PLEXUS.

The double recessive black plexus was easily obtained, and a back-cross test was made of the amount of crossing-over in the female between these two loci (table 99). The back-cross cultures furnished 1,026 flies, of which 417 or 40.6 per cent were cross-overs. This very free crossing-over located plexus in the region of arc, if its locus were to the right of black, or at a point even farther to the left than streak if it were to the left of black.

TABLE 99.—*P_1, black plexus ♀ × wild ♂; B. C., F_1 wild-type ♀ × black plexus ♂.*

Jan. 1, 1915.	Black plexus.	Wild-type.	Black.	Plexus.
1084.....	107	84	68	42
1085.....	58	58	47	51
1086.....	100	111	62	59
1099.....	48	43	45	43
Total....	313	296	222	195

A means of easily testing these alternatives was soon afforded by the discovery and location of "star," which proved to be a dominant mutation whose locus is some distance to the left of streak. A star black plexus back-cross was made by testing the F_1 star daughter, from the mating of a star female by a black plexus male, to black plexus males (table 100). Four such pair cultures gave 1,352 offspring, of which 233 were double cross-overs, 343 simple cross-overs between black and plexus, 289 single cross-overs between star and black, and 487 were non-cross-overs. Plexus gave 42.6 per cent of total observed

crossing-over with black and 46.7 per cent with star, from which it was known that the locus of plexus is to the right of black. A comparison of the combined black plexus cross-over value of 41.8 with other values involving black indicated that the locus of plexus was closest to arc, but probably not quite as far to the right, since black and arc gave 42.6 per cent of observed crossing-over.

TABLE 100.—P_1, *black plexus* ♂ × *star* ♀; *B. C.*, F_1 *star* ♀ × *black plexus* ♂.

July 20, 1915.	$\frac{S'}{b \quad p_z}$		$\frac{S' \mid b \quad p_z}{\mid \quad \mid}$		$\frac{S' \mid \quad p_z}{b \mid \quad}$		$\frac{S' \mid b \mid}{\mid \quad \mid p_z}$	
	Star.	Black plexus.	Star black plexus.	Wild-type.	Star plexus.	Black.	Star black.	Plexus.
1921.....	81	81	42	48	43	52	32	21
1922.....	65	41	24	42	45	42	36	28
1923.....	57	52	35	39	42	40	29	43
1924.....	72	38	22	37	36	43	23	21
Total....	275	212	123	166	166	177	120	113

The loci of plexus and arc were found to be so close together that it was too hard a task to obtain the plexus arc double forms by any simple method. The plexus speck double was obtained fairly easily.

TABLE 101.—*Summary of all crossing-over data on plexus.*

Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Star plexus..	1,352	632	46.7	July 20, 1915	Bridges	p_z; $\frac{S'}{b \quad p_z}$ B. C.; 1921–'24.
Squat plexus.	82	39	47.6	April 3, 1916	Do.	S_q; $\frac{S_q}{b \quad p_z}$ B. C.; 4044.
Black plexus.	1,026	417	40.6	Jan. 1, 1915	Do.	p_z; $b \; p_z$ B. C.; 1084–'99.
	1,352	576	42.6	July 20, 1915	Do.	p_z; $\frac{S'}{b \quad p_z}$ B. C.; 1921–'24.
	82	38	46.4	Apr. 3, 1916	Do.	S_q; $\frac{S_q}{b \quad p_z}$ B. C.; 4044.
	2,460	1,031	41.9			
Purple plexus.	344	164	47.7	Feb. 29, 1916	Do.	*IIIa*; $p_r \; p_z \; s_p$ F_1; 3535–'53.
Plexus speck..	327	29	8.9	Feb. 29, 1916	Do.	*IIIa*; $p_r \; p_z \; s_p$ F_1; 3535–'53.

Only one linkage experiment involving the plexus speck cross-over value is available, and this gave 8.9 per cent of crossing-over (table 135). The locus of plexus is therefore about 3 units to the left of arc, since arc gave 5.9 per cent of crossing-over with speck.

This position makes plexus of great importance, since it can serve as a new base of reference for speck itself. At present speck is located by reference to curved, which is too remote to give an accurate measure

of the interval, especially since the amount of double crossing-over and of coincidence in this region are known only by inference from other experiments, there being no intermediate locus by means of which direct calculation could be made. Arc could be used for this purpose, but is unsuitable because of a probable confusion in classification with curved. Plexus, on the other hand, being a venation character only, can readily be used with curved, and its position is more favorable than arc, since it more nearly divides the gap.

Preparations were made to make an extensive experiment which should give data on the plexus speck distance as well as on several others throughout the length of the chromosome. But as yet this has not proceeded further than the synthesis of the multiple recessive needed (d b p_r c p_x s_p), and of the two parent stocks necessary for an "alternated" experiment (S′ b c s_p and d p_r p_x).

A summary of the crossing-over data imvolving plexus is given in table 101. The locus of plexus is about 8.9 units to the left of speck, or at 96.2.

VALUATION OF PLEXUS.

Sturtevant has reported trouble in the classification of plexus in certain crosses, and it is not certain that all the plexus individuals can be separated from wild-type where the variation is great. In none of the experiments here reported was difficulty encountered.

LIMITED.

(Fig. 81.)

In carrying out the black arc morula back-crosses (culture 513, September 13, 1914), Bridges noticed that there was present a character somewhat similar to abnormal abdomen, except that its main effect was evident on the ventral surface of the abdomen and to a slight extent on the side. The chitinous ventral plates on the abdomen, instead of being full size with rounded edges and many regularly arranged small hairs (as in fig. 81), were reduced often to half the size by an irregular erosion of the edges.

TEXT-FIGURE 81.—Limited bands to the abdomen.

The color also was etched. The hairs were very few in number, and those irregularly arranged and directed. The dorsal plates where they bent around to the ventral side were affected in the same manner at their ends, though not so strikingly.

This character was almost entirely limited to the morula flies and was distributed in such a way that its locus was certainly second-chromosome and probably to the right of morula, though the counts were not made carefully enough to be sure of this.

The black arc morula stock was found to be showing the limited band character in all or nearly all of the morula flies, and this condition has been maintained for some five years, which means that the linkage is very close. It is in fact not entirely certain that limited may not be found to be still another effect of morula itself.

CONFLUENT (C_f).

ORIGIN OF CONFLUENT.

In culture 550 (which was part of the tests of the method of transmission of the ability to produce exceptions by non-disjunction), Bridges found a single male which had thickened, knotted veins in the wing (September 23, 1914). The vein most thickened was the second longitudinal opposite the anterior cross-vein, but especially at the tip, where it was confluent for quite a space with the marginal vein. In addition both cross-veins were thickened and irregular. The wing as a whole was slightly smaller than usual and the fly seemed rather sluggish.

INHERITANCE OF CONFLUENT.

This male was out-crossed to a wild female and in F_1 produced nearly half of the offspring with confluent veins (culture 592, table 102). From this F_1 result confluent was known not to be sex-linked, since the characters appeared in half the F_1 males as well as females, instead of in none of the males and all of the females, as it would have done had it been a sex-linked dominant (like bar).

TABLE 102.—*Confluent* ♂ (*heterozygous*) × *wild* ♀.

Oct. 5, 1914.	Wild-type ♀.	Wild-type ♂.	Confluent ♀.	Confluent ♂.
592.....	24	20	17	11
627.....	52	33	38	47
628.....	42	41	37	33
901.....	41	37	36	46
1038.....	10	11	12	14
Total....	169	142	140	151

Some of the F_1 confluent males were again out-crossed to wild females, and all the cultures of similar character (table 102) gave a total of 600 flies, of which 291 or 48.5 per cent were confluent. Thus the viability of confluent is not bad, though the sterility is very high. Very many such matings failed, and this was especially true of the confluent by confluent matings.

The confluent by confluent matings gave consistently about two-thirds of the flies confluent and one-third wild-type. This suggested that, like streak, confluent was lethal when homozygous.

The stock was run by mass-cultures of confluent by confluent, and these also gave the same percentages of confluent. The stock was maintained (with difficulty) for two years by this method and there was no indication of an increase in the percentage of confluent, though no counts were made. These facts prove that homozygous confluents either die, as supposed, or else play only a negligible rôle through sterility if they occasionally survive.

TABLE 103.—*P_1, confluent ♂ × purple curved speck ♀ or × sepia peach ebony ♀.*

Oct. 23, 1914.	Wild-type ♀.	Wild-type ♂.	Confluent ♀.	Confluent ♂.
629.....	6	3	6	8
732.....	10	5	16	11
716.....	5	8	5	2
717.....	40	25	28	17
Total.....	61	41	55	38

CHROMOSOME OF CONFLUENT.

Confluent males were out-crossed to purple curved speck females (cultures 629 and 732, table 103) and to sepia-peach-ebony females (cultures 716 and 717), as P_1 matings for male back-cross tests to determine the linkage relation of confluent to the second and to the third chromosomes respectively. The tests were made with great difficulty, owing to the sterility and low productivity of confluent. The second-chromosome tests gave a total of 71 offspring, none of which was a cross-over between confluent and any of the three second-chromosome loci (table 104). The third-chromosome tests gave recombination between confluent and the third-chromosome loci, although as soon as the results of the second-chromosome tests became apparent these third-chromosome counts were discontinued.

TABLE 104.—*B. C., confluent F_1 ♂ (from table 103) × purple curved speck ♀.*

Nov. 26, 1914.	Confluent.	Purple curved speck.
802.....	11	2
840.....	2	3
841.....	3	5
1149.....	6	19
1150.....	12	8
Total.....	34	37

VALUATION OF CONFLUENT.

The very low productivity and high sterility of confluent made it evident that there was little use to be obtained from the mutant in spite of its dominance, good viability, and perfect separability. The

determination of the locus was not made, though this would have been done had the stock not died out because of its low productivity and sterility.

CONFLUENT *VIRILIS*.

Metz (C. W. Metz, Journ. Gen., 1916, p. 591) found in the species *Drosophila virilis* a mutation which was a very striking counterpart of confluent of *D. melanogaster* in all respects, save that it was neither so sterile nor so non-productive. The character of the venation was practically the same in the two cases, though in confluent *D. melanogaster* the venation may have been a trifle thicker and knottier in the affected regions. Confluent *D. virilis* was a dominant which gave 1 : 1 ratios upon inbreeding, precisely as did confluent *D. melanogaster*.

There is no doubt of the completely lethal effect of confluent *virilis* when homozygous, and in confluent *melanogaster* the only indication that an occasional homozygote may survive is the fact that 1 out of 10 of the flies successfully tested by Metz gave a 27 : 0 ratio of confluent to wild-type, instead of the 18 : 9 ratio expected. The other 9 flies tested by Metz were all heterozygous, as had been all those worked with by Bridges. It is possible that this 27 : 0 ratio was the result of a balanced lethal condition such as obtains in truncate, snub, beaded, and other stocks.

The fact that several of the mutations secured in *D. virilis* (or other species) seem parallel in appearance and inheritance with the known mutants of *D. melanogaster* is of great interest as an indication of the basic similarity of the two systems of genetic materials.

FRINGED (*f*,).

(Text-figure 82.)

ORIGIN OF FRINGED.

In the F_2 from a cross of the sex-linked wing-character "jaunty I" to wild (culture 1042, January 20, 1915), Bridges found that about a quarter of the flies of both sexes were showing an irregular distribution of the hairs on the marginal vein of the wing. The margin showed spots entirely denuded of hairs or with only weak hairs, while the remaining hairs were frayed and irregular in directions. The wings also were slightly smaller, a trifle discolored, and occasionally divergent.

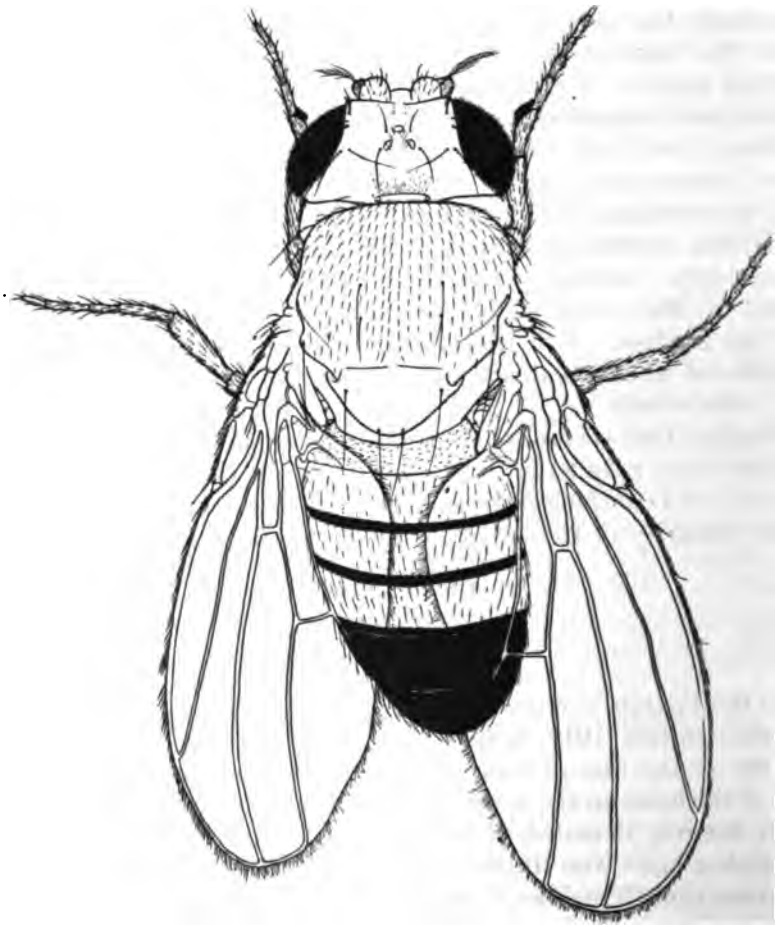
CHROMOSOME CARRYING FRINGED.

One of the "fringed" males was out-crossed to a black female and produced in F_2 the typical 2 : 1 : 1 : 0 ratio that showed that the locus of fringed is in the second chromosome (table 105). From the F_2 black and fringed inbred a stock of black fringed was obtained in F_4.

A similar attempt to obtain a fringed speck double-recessive stock from the F_2 of the cross of fringed by speck (table 106) failed entirely.

LOCUS OF FRINGED.

The black fringed stock, in combination with the recently mapped dominant star, offered a means of locating the position of fringed. A three-locus back-cross was started by mating a black fringed male to a star female and back-crossing the F_1 star females by black fringed males. The three back-cross cultures (table 107) gave a total of 496 flies, of which 153 were non-cross-overs, 133 single cross-overs between star and black, 141 single cross-overs between black and fringed, and



TEXT-FIGURE 82.—Fringed wing-margin.

70 were double cross-overs. The black fringed cross-over value was 42.5, which places fringed at practically the same locus as arc, which gave 42.6 as the black arc cross-over value.

To determine the locus more closely than this would require fringed-speck or fringed arc back-crosses, which have not been made. The only cross-over data on fringed are the values calculated from the star black fringed back-cross above, viz, $S'b = 40.9$, $b\,f_r = 42.5$, $S'f_r = 55.2$.

In the spring of 1915 Morgan also found fringed, probably through use of the same wild stock from which it originally came in the cross to jaunty I.

TABLE 105.—P_1, *fringed* ♂ × *black* ♀; F_1 *wild-type* ♀ + F_1 *wild-type* ♂.

Feb. 13, 1915.	Wild-type.	Black.	Fringed.	Black fringed.
1361.....	216	100	96	0
1362.....	150	52	56	0
Total....	366	152	152	0

TABLE 106.—P_1, *fringed* ♂ × *speck* ♀; F_1 *wild-type* ♀ + F_1 *wild-type* ♂.

Feb. 23, 1915.	Wild-type.	Fringed.	Speck.	Fringed speck.
1363.....	112	61	64	0
1364.....	165	58	94	0
Total....	277	119	158	0

TABLE 107.—P_1, *star* ♀ × *black fringed* ♂; *B. C.*, F_1 *star* ♀ × *black fringed* ♂.

Oct. 23, 1915.	S' / b f_r		S' / b f_r		S' / f_r / b		S' b / / f_r	
	Star.	Black fringed.	Star black fringed.	Wild-type.	Star fringed.	Black.	Star black.	Fringed.
2282.....	36	33	24	28	20	26	13	15
2283.....	33	12	14	20	27	16	2	11
2284.....	24	14	19	28	19	33	16	13
Total.....	93	59	57	76	66	75	31	39

STAR (*S'*).

(Text-figure 83.)

ORIGIN OF STAR.

In an experiment by means of which it was proved that the exceptional sons produced through secondary non-disjunction are themselves unable to transmit the power of producing further secondary exceptions (Bridges, 1916, p. 44), an eosin sable forked male was found which had an eye of the moruloid type and which was very similar in appearance to the sex-linked mutation "facet" (culture, 1347, February 12, 1915).

INHERITANCE OF STAR.

It was assumed that this character was sex-linked, since it had appeared in a single male in a pair culture, as is usual with sex-linked mutations. For this reason the matings for F_2 were made without

examining the character of the F_1 flies, and it was not until the F_2 began to hatch that it was realized that the other alternative was correct—that "star," as the character was called, was an autosomal dominant. Two of the F_1 pairs gave in F_2 no star whatever (1627, 1629), while a third pair (1628, table 108) gave stars among both males and females to the extent of half the flies (52 per cent). The fact that half the flies were stars showed that this culture came from a heterozygous dominant and a wild-type F_1 pair. That star was an autosomal dominant was proved by the sister cultures which gave no stars; had star been sex-linked all the F_1 females would have been star and hence every F_2 pair should have given results like those of culture 1628.

TEXT-FIGURE 83.—Star eye, showing the arrangement of the facets and hairs. Compare with the normal condition shown in plate 10, figure 3c.

These facts were confirmed by the results of further tests of star males; for star males out-crossed to wild females gave in F_1 stars to the extent of half the flies (table 109, 337 stars in a total of 683, or 49.3 per cent), and the stars were evenly distributed among the males and females. Had star been sex-linked, none of the males but all of the females should have been star.

TABLE 108.—P_1, *star* ♂ × *wild* ♀; F_1 *pair* (F_1 *flies chosen at random*).

Mar. 12, 1915.	Wild-type ♀.	Star ♀.	Wild-type ♂.	Star ♂.
1628.....	44	39	33	45

TABLE 109.—P_1, *star* ♂ × *wild* ♀.

Mar. 27, 1915.	Wild-type ♀.	Wild-type ♂.	Star ♀.	Star ♂.
1719.....	129	136	129	115
1914.....	28		24	
1915.....	27		32	
1916.....	26		37	
Total....	346		337	

LETHAL NATURE OF THE HOMOZYGOUS STAR.

At the same time that the male out-crossed tests were made, a few pairs of star female by star male were mated. In the next generation, which corresponded to an F_2, the flies in one culture (1739, table 110) were exactly two-thirds star and one-third wild-type, which is the typical yellow-mouse ratio that had already been met with in *Drosophila* in the case of streak. The other culture (1740, table 110) gave nearer to a 3 to 1 ratio. Further matings were necessary to be sure

which ratio was really present. These further cultures left no doubt that the ratio was really the 2 : 1 ratio corresponding to a dominant lethal when homozygous. The total number of stars in such cultures was 766, which is 67.3 per cent of the total number, where 66.7 per cent are expected according to the lethal assumption.

TABLE 110.—*F_1 star* ♀ + *F_1 star* ♂.

Apr. 9, 1915.	Wild-type.	Star.
1739.....	58	117
1740.....	25	71
1877.....	46	91
1878.....	29	77
2025.....	30	62
2026.....	71	139
7454.....	100	166
7455.....	12	43
Total.....	371	766

TABLE 111.—*P_1, star* ♂ × *peach sooty* ♀.

Mar. 31, 1915.	Wild-type ♀.	Wild-type ♂.	Star ♀.	Star ♂.
1730.....	48	39	43	47

B C., F_1 star ♀ × peach sooty ♂.

Apr. 12, 1915.	Not-star.				Star.			
	p^p e^s		p^p / e^s		p^p e^s		p^p / e^s	
	Peach sooty.	Wild-type.	Peach.	Sooty.	Star peach sooty.	Star.	Star peach.	Star sooty.
1745.....	19	11	3	1	16	6	5	5
1746.....	6	3	1	3	6	6	2	2
1747.....	16	17	4	8	15	16	2	4
1748.....	4	4	2	1	8	8	2	3
1749.....	6	10	6	3	5	4	3	2
1750.....	18	27	9	14	17	19	10	5
Total.....	69	72	25	30	67	59	24	21

The history of the stock likewise proved the lethal nature of the homozygote; for star flies were inbred for many generations in mass-culture without giving any closer an approach to a pure-breeding stock. Likewise none of the star flies selected for out-crossing on very numerous occasions ever proved to be homozygous; all gave the 1 : 1 ratio typical of a heterozygous dominant. Lately, much more vigorous tests have conclusively proved that star is lethal when homozygous.

CHROMOSOME CARRYING STAR.

To test the relation of star to the third chromosome, a star male was out-crossed to the double-recessive peach sooty (peach is an allelomorph of pink, and sooty an allelomorph of ebony). In F_1 the flies were, as expected, half stars and half wild-type (culture 1730, table 111).

Some of the F_1 star females were back-crossed by peach sooty males (table 111). With two loci as far apart as peach and sooty were known to be, there was no need to run a male back-cross test, since the female test must readily reveal linkage to one or to the other of these two loci if the tested gene is in the third chromosome. As a matter of fact, there was complete independence of star and peach (52.6 per cent of recombination) and also of star and sooty (50.4 per cent of recombination). Peach and sooty gave 27.3 per cent of crossing-over, which is a trifle higher than the usual value.

LOCUS OF STAR.

Since the locus of star was proved not to be in the third chromosome, the chances were about 50 to 1 that its locus was in the second chromosome. This probability was so great that an extensive experiment was planned and started without the relation to the second chromosome having been previously tested. This experiment was the quadruple

TABLE 112.—P_1, *star* ♀ × *purple curved speck* ♂.

June 28, 1915.	Star speck.	Wild-type.	Star.	Speck.
1806.....	45	51	40	50
1807.....	42	47	43	51
Total....	87	98	83	101
1808.....	.....	83	95	.....

back-cross of star and purple curved speck, which was to serve several purposes. In the first place, it was to give an accurate measure of the amount of crossing-over between curved and speck, which was very important, since up to that time only a relatively small amount of data was available on this value whereby the locus of speck and with it the entire right end of the chromosome was mapped in relation to the rest; in the second place, it was to establish the locus of star, which, as was then realized, might prove to be the most useful of all the second-chromosome characters. These linkage values were both to be controlled and linked up by means of the accurately mapped loci purple and curved. The third purpose was to test more thoroughly the extent and nature of the change of crossing-over with age in different broods and in different regions of the second chromosome, but more especially the relation between this change and the change in the amount of coincidence (see Bridges, 1915).

When the F_1 flies from the cross of star by purple curved speck began to hatch, a surprise was met with in that half of the flies were speck in two cultures (1806, 1807), but not in the third (1808, table 112). It had not been noticed before that there was any speck in the star stock; and it is not clear how speck came to be there, since no cross to speck had been made, and so far as known none of the stocks concerned in the history of star had contained speck even as an impurity. However, this circumstance gave an immediate test of the linkage relation of star and speck, since these two cultures constituted a star speck back-cross test of crossing-over in the female. The two cultures gave 369 flies, of which 184, or 49.9 per cent, were cross-overs. While this value is that corresponding to any locus as far from speck as black, or any other to the left of black, it is also the result one would obtain if star were not in the second chromosome at all. This possibility caused such concern for the experiment already planned that imme-

TABLE 113.—*F_1, star speck ♂ × purple curved speck ♀.*

July 19, 1915.	Star speck.	Purple curved speck.	Star curved speck.	Purple speck.
1913.....	60	73	1	0

diately a black-cross test of crossing-over in the male between star and purple curved speck was carried out. This male test proved that star is actually in the second chromosome, since of the 134 flies (culture 1913, table 113) 133 were non-cross-overs, as opposed to the very free crossing-over in the female test.

CROSSING-OVER IN THE MALE.

But one fly was a *cross-over*, since it was distinctly a star *curved* speck male, while all other flies were either star speck or purple curved speck. This cross-over fly occurred on the sixth day of the counts of a pair culture, so that there is no possibility of overlap of generations; and no opportunity for contamination, since no possibility of star curved flies had ever existed in any other or previous culture. That classification and pedigree were both as recorded was proved by a test

TABLE 114.—*Cross-over star curved speck ♂ × purple curved speck ♀.*

Aug. 7, 1915.	Star curved speck.	Purple curved speck.
2048.....	26	29

made with the cross-over male. He was out-crossed to a purple curved speck female and produced star curved speck offspring in equal numbers (culture 2048, table 114). There can be little doubt,

then, that in this case there had been crossing-over *in the male* between the loci purple and curved. It is true that there are two or three possible escapes from this necessity. Thus, "deficiency" for the curved locus occurring in the star speck gamete of the father would give precisely this result. Likewise, mutation to curved occurring in the germ-tract of the star speck male would give this result. "Duplication" of the curved locus in the purple curved speck mother would answer as well. All three of these processes have been met with and amply established elsewhere in *Drosophila*. (See especially Bridges, 1917, Genetics, 2, p. 454.)

Two of these alternative explanations, deficiency and duplication, were capable of differentiation from the other two and from each other by proper tests, but these were not made. There was one previously well-established case of crossing-over in the male (Muller, 1916), but this occurred in a very early embryonic stage and hence affected all the gametes. It is to be doubted if even the case just described is to be considered as brought about by a mechanism analogous to that by which crossing-over in the female is regularly effected.

As the first broods of the star purple curved speck back-cross began to hatch it became apparent that the position of star is very far to the left of purple—even further than streak. The completed counts (table 115) showed that star and purple gave a total of 3,010 cross-overs in the 6,766 flies, or 44.5 per cent of crossing-over. Streak had given only 33.1 per cent of crossing-over with purple, so that when allowance was made for double crossing-over, star was calculated at a position fully 16 units to the left of streak. With this addition to the total length of the map of the second chromosome, it was about 110 units, or very nearly twice as long as the X-chromosome map.

The crossing-over between curved and speck proved to be slightly greater than the previous data had indicated, for there was 30.5 per cent of observed crossing-over between curved and speck. The total available data (table 115) gave 3,037 cross-overs in a total of 10,042 flies, or 30.2 per cent. When a correction is made for double crossing-over according to the probable coincidence of 20, the locus of speck is found to be about 31.6 units to the right of curved or at 105.1.

With respect to the third problem involved, that of the relation of age to the amounts of crossing-over and of coincidence, these data proved rather unsuitable, because of the large interval between star and the other three loci. Because of this fact none of the intervals worked with were short enough to exclude double crossing-over and furnish an uncomplicated measure of the effects of the age change. This demerit was partly compensated by the fact that nearly the entire length of the chromosome was covered by the four loci.

In general, these cultures and their totals showed the usual drop in the cross-over values of the second broods (table 116) and a slight

TABLE 115.—P_1, *star* ♀ × *purple curved speck* ♂; *B. C.*, F_1 *star* ♀ × *purple curved speck* ♂.

July 11, 1915.	S' p_r c s_p		S' | p_r c s_p		S' p_r | c s_p		S' | s_p p_r c		S' p_r | c s_p		S' p_r c | s_p		S' | c p_r | s_p		S' p_r | s_p | c		
Firsts.	Star.	Purple curved speck.	Star purple curved speck.	Wild-type.	Star curved speck.	Purple.	Star speck.	Purple curved.	Star purple.	Curved speck.	Star purple curved.	Speck.	Star curved.	Purple speck.	Star purple speck.	Curved.	Total.
1836.....	14	16	9	18	5	12	12	13	10	2	8	10	.....	2	2	1	134
1837.....	35	40	22	21	22	13	17	14	13	10	11	15	3	3	1	.....	240
1838.....	46	47	25	45	13	14	24	15	15	15	19	19	3	2	.....	1	303
1839.....	20	20	10	15	3	12	13	7	5	.....	11	13	1	1	2	.....	133
1840.....	32	21	19	26	8	8	11	17	6	4	12	13	1	2	.....	1	181
1841.....	22	15	13	12	4	3	11	9	4	6	7	11	1	2	.....	1	121
1842.....	47	44	31	27	9	16	21	17	11	19	18	18	4	3	2	1	288
1843.....	65	31	29	40	18	23	28	23	16	9	17	18	6	3	1	3	330
1844.....	34	31	27	24	8	3	19	19	7	13	11	12	2	1	.....	1	212
1845.....	15	15	12	11	.....	7	10	9	10	2	4	13	.....	1	.....	1	110
1846.....	33	30	26	25	13	8	19	10	9	10	16	13	8	.....	2	.....	222
1847.....	61	51	27	42	17	12	15	24	9	22	17	18	9	6	1	2	333
1848.....	33	33	30	36	18	11	18	21	13	13	26	18	2	2	6	1	281
1856.....	57	34	34	25	10	20	24	15	8	14	16	11	2	.....	3	4	277
1857.....	59	54	33	50	19	24	30	22	21	22	16	33	3	1	3	4	394
1858.....	43	41	32	33	17	15	23	26	24	17	15	16	1	1	1	1	306
1859.....	34	26	30	27	16	23	20	20	15	20	22	21	6	5	2	6	293
1860.....	44	48	52	48	19	16	26	20	16	17	24	20	4	7	3	2	366
1869.....	53	50	34	38	18	11	34	30	18	12	19	22	2	5	3	2	351
1870.....	57	45	37	35	17	14	23	30	14	9	23	22	3	2	2	2	335
1871.....	81	63	47	48	28	26	47	28	14	17	20	26	2	5	.....	.....	452
1872.....	34	30	20	23	8	14	14	11	8	6	9	14	1	3	2	1	198
1873.....	38	42	38	30	15	21	22	20	8	8	18	23	2	1	.....	4	290
1890.....	44	34	33	35	12	11	32	31	14	13	19	18	3	2	2	3	306
1893.....	23	25	20	28	7	13	15	16	11	11	14	11	2	1	2	1	200
1894.....	7	12	20	15	4	9	5	5	7	4	10	7	2	1	1	1	110
Total....	1,031	898	710	777	328	359	533	472	306	295	402	435	73	62	41	44	6,766

TABLE 116.—*Second 10-day broods from same parents as table 115.*

July 21, 1915.		S' / p_r c s_p		S' / p_r c s_p		S' / c s_p / p_r		S' / s_p / p_r c		S' / p_r / c s_p		S' / p_r c / s_p		S' / c / p_r / s_p		S' / p_r / s_p / c		Total.
Firsts.	Seconds.	Star.	Purple curved speck.	Star purple curved speck.	Wild-type.	Star curved speck.	Purple.	Star speck.	Purple curved.	Star purple.	Curved speck.	Star purple curved.	Speck.	Star curved.	Purple speck.	Star purple speck.	Curved.	
1836	1925	27	16	10	23	11	5	9	8	2	8	9	10	3	1	1	.....	143
1838	1926	77	61	31	41	15	11	26	25	6	6	12	19	1	1	.....	2	334
1839	1927	41	38	32	33	12	10	23	20	8	6	16	19	.....	1	1	.....	260
1840	1928	23	29	17	20	9	13	15	21	11	7	3	12	1	.....	.....	.....	181
1841	1929	41	30	24	29	5	9	15	15	2	9	9	12	1	.....	.....	.....	201
1842	1930	59	66	30	32	16	8	30	22	5	18	15	18	.....	1	2	.....	322
1843	1931	38	36	30	34	16	16	18	19	7	9	15	15	1	2	2	1	259
1845	1932	33	34	13	22	11	8	8	13	3	9	11	11	1	.....	1	2	180
1846	1933	26	21	23	27	8	6	10	15	8	9	10	14	.....	2	1	.....	180
1847	1934	5	10	2	6	1	1	2	3	1	1	.....	3	1	.....	.....	.....	36
1848	1935	49	40	29	33	10	12	22	21	9	13	15	19	1	2	.....	2	277
1856	1936	45	36	37	41	17	17	26	20	16	11	26	19	2	2	.....	.....	315
1857	1937	42	31	31	23	27	16	18	15	8	9	14	15	3	.....	.....	4	256
1858	1938	44	49	26	27	14	19	21	20	12	6	11	16	3	4	2	2	276
1859	1939	29	40	28	27	13	12	10	17	6	14	18	18	3	5	1	2	243
1860	1940	53	45	34	48	11	15	19	17	9	12	17	15	1	1	3	2	302
1869	1957	49	43	25	41	18	10	28	21	10	8	22	17	4	4	2	4	306
1870	1958	56	52	33	28	11	15	27	33	9	5	18	14	1	1	1	1	305
1871	1959	43	42	31	33	13	12	27	16	6	11	12	18	1	1	1	2	269
1872	1960	38	42	15	17	12	2	13	18	3	4	15	9	1	.....	1	.....	190
1873	1961	32	32	33	24	11	11	17	16	2	7	10	12	1	2	2	.....	212
1893	1967	17	21	6	13	6	6	12	8	4	3	3	11	.....	1	.....	.....	111
1894	1968	18	17	11	11	3	3	8	14	4	6	4	17	.....	1	.....	.....	117
Total....		885	831	551	633	270	237	404	397	151	191	285	333	30	32	21	24	5,275

TABLE 117.—*Third broods from same parents as table 115.*

July 31, 1915.		S′ p_r c s_p		S′ p_r c s_p		S′ p_r c s_p		S′ p_r c s_p		S′ p_r c s_p		S′ p_r c s_p		S′ p_r c s_p		S′ p_r c s_p		S′ p_r c s_p		Total.
Seconds.	Thirds.	Star.	Purple curved speck.	Star purple curved speck.	Wild-type.	Star curved speck.	Purple.	Star speck.	Purple curved.	Star purple.	Curved speck.	Star purple curved.	Speck.	Star curved.	Purple speck.	Star purple speck.	Curved.		
1929	1982	42	40	18	29	8	7	11	15	7	4	9	8	1	1	1	.....		201
1930	1983	34	34	20	18	10	8	14	18	2	5	5	7	1	.....	.....	.....		176
1932	1984	13	11	7	10	4	5	2	5	3	.....	.....	3	2	.....	.....	.....		65
1935	1985	31	27	22	29	13	12	20	25	6	3	14	16	1	.....	1	1		221
1936	1986	14	12	8	7	5	1	6	7	3	3	3	5	.....	1	.....	.....		75
1937	1987	6	12	5	3	4	3	5	5	2	.....	9	6	.....	.....	.....	.....		60
1938	1988	46	43	21	31	15	11	20	25	10	10	16	23	2	4	1	1		279
1939	1989	19	23	34	24	9	9	14	14	9	4	11	9	1	2	1	2		185
1940	1990	37	51	32	32	14	15	23	20	9	10	26	16	1	2	3	2		293
1957	2034	32	40	21	27	12	13	16	19	8	9	14	15	5	.....	1	3		235
1959	2035	42	28	18	43	8	9	14	9	10	6	13	12	1	.....	1	.....		214
1960	2036	40	50	25	14	5	11	21	23	6	3	18	11	.....	2	.....	1		230
1961	2037	14	12	6	8	6	3	5	4	1	1	3	3	1	.....	.....	.....		67
Total....		370	383	237	275	113	107	171	189	76	58	141	134	16	12	9	10		2,301
Thirds.	Fourths.	Fourth broods from same parents as table 115; Aug. 10, 1915.																	
1982	2052	11	17	7	8	5	1	3	10	3	3	3	5	.....	.....	1	.....		77
1985	2054	15	7	5	12	4	3	5	7	2	4	2	1	1	.....	.....	1		69
1989	2053	6	3	3	4	1	.....	3	4	1	3	1	.....	.....	1	.....	.....		30
2036	2064	7	13	1	6	1	1	2	1	1	3	.....	.....	.....	.....	.....	.....		36
Total....		39	40	16	30	11	5	13	22	7	13	6	6	1	1	1	1		212

continued drop for the third and fourth broods (table 117). The only significant deviation from this rule is in the case of curved speck, which rose slightly in the second broods (table 118). This probably means that the normal fall was more than compensated for by the concomitant change in the amount of double crossing-over in this particular region. The coincidence values for the experiment show a very slight drop with age, but here again the intervals are so long that conciseness is lost and the real effect on coincidence obscured (table 119).

TABLE 118.—*Crossing-over values for successive broods of the star-purple curved speck back-cross.*

Interval.	Firsts.	Seconds.	Thirds.	Fourths.
Star purple.....	44.5	41.5	40.8	37.8
Purple curved..	22.3	18.1	17.4	18.9
Curve dspeck...	30.5	34.6	29.8	24.1

TABLE 119.—*Coincidence values for different regions of the star purple curved speck back-cross.*

Regions.	Firsts.	Seconds.	Thirds.
S'–pr pr–c..	102.2	96.5	93.5
pr–c c–sp...	47.8	38.7	39.3
S'–pr c–sp..	100.3	104.5	104.9
S'- pr–c–sp...	41.6	39.2	39.0

TABLE 120.—*P_1, star ♀ × dachs ♂; B. C., F_1 star ♀ × dachs ♂ from stock.*

Sept. 15, 1915.	Star.	Dachs.	Star dachs.	Wild-type.	Total.
2146.....	90	47	17	24	178
2147.....	49	64	18	15	146
2148.....	76	60	19	20	175
2149.....	66	61	20	29	176
2150.....	43	18	5	7	73
2218.....	67	74	23	33	197
2219.....	36	31	17	21	105
2220.....	63	55	17	20	155
2221.....	84	51	15	29	179
2305.....	82	75	35	41	233
Total....	656	536	186	239	1,617

The star purple curved speck back-cross did not allow of a close calculation of the locus of star because of the great distance between star and purple, with little knowledge of the amount of coincidence involved. For this reason it was sought to use dachs as the base of reference for star, since dachs is about 23.5 units nearer star than purple is. Streak was known to be even nearer, but the classification

of streak was then in disfavor (through its failure in the streak dachs back-cross), so that dachs seemed more practical.

A star dachs back-cross furnished 1,617 flies, of which 425, or 26.3 per cent, were cross-overs (table 120). Several other experiments have been made which indirectly gave data on this interval. The total available data (table) furnish 3,472 flies, of which 949 or 27.3 per cent were cross-overs. When allowance for double crossing-over corre-

TABLE 121.—P_1, *star purple* ♀ × *streak dachs* ♂; *B. C.*, F_1 *star streak* ♀ × *dach spurple* ♂.

Aug. 24, 1916.	S' / S_k	p_r / d	S' / S_k d	p_r	S' / S_k	d / p_r	S' / S_k d	p_r
	Star purple.	Streak dachs.	Star streak dachs.	Purple.	Star dachs.	Streak purple.	Star.	Streak dachs purple.
4999	36	21	3	4	10	10	17	8
5024	21	15	3	5	5	.....	12	4
5025	31	13	6	12	8	4	10	4
5036	24	26	7	3	.....	5	8	6
5040	52	29	7	13	12	9	11	3
5041	14	14	4	4	4	3	7	5
5042	20	15	6	6	3	4	8	5
5043	24	24	7	16	2	6	7	3
5055	26	13	5	4	3	2	3	3
5110	71	51	7	12	10	10	17	8
Total	319	221	55	79	57	53	100	49

Aug. 24, 1916.	S' / S_k / d	p_r	S' / S_k d	p_r	S' / S_k	d / p_r	S' / S_k /	d / p_r	Total.
	Star streak purple.	Dachs.	Star streak dachs purple.	Wild-type.	Star dachs purple.	Streak.	Star streak.	Dachs purple.	
4999	.....	16	.....	2	4	2	.....	10	143
5024	.....	.....	.....	1	.....	1	.....	.....	67
5025	1	4	3	2	.....	1	.....	2	101
5036	.....	.....	1	.....	.....	.....	.....	.....	80
5040	.....	18	.....	.....	1	.....	.....	4	159
5041	.....	2	1	.....	.....	.....	.....	1	59
5042	.....	5	1	1	.....	1	.....	2	76
5043	.....	.....	.....	2	.....	1	.....	.....	92
5055	.....	1	.....	1	.....	.....	.....	.....	61
5110	.....	.....	1	.....	.....	1	.....	1	189
Total	1	46	7	9	5	6	.....	20	1,027

sponding to the probable coincidence of 30 is made, the locus of star is indicated as 28.8 units to the left of dachs or 46.5 units to the left of black.

As the usefulness of star developed, there was greater necessity for a still more accurate mapping of the star locus. More recent work with streak had shown that tolerably accurate results even under

unfavorable conditions could be obtained with streak, especially as long as the calculations are restricted to the flies which actually show the streak character above a certain definite grade of development. A back-cross was therefore undertaken between star and streak, between streak and dachs, and between dachs and purple—all in the same experiment. The advantage of purple is that its locus is securely mapped in the main body of loci, and its presence serves as the con-

TABLE 122.—*Summary of all cross-over data involving star.*

Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Star streak..	396	63	15.9	Aug. 24, 1916	Bridges	$S'; \frac{S' \quad pr}{S_k \, d}$ B. C., *Sk* flies only; 4999–5110.
Star cream L	389	86	22.1	Oct. 20, 1916	Do.	$c_{rb}; \frac{S'}{crb}$ B.C.; 5593+5824.
Star truncate	549	149	27.1	May —, 1917	Wallace	*Snub*; p. 143, this paper.
Star dachs...	96	31	32.3	Sept. 12, 1915	Do.	$d_l; \frac{S'}{d}$ F_2, dachs flies; 2141–2216.
	152	53	34.8	Sept. 12, 1915	Do.	idem, not-dachs flies.
	1,617	425	26.3	Sept. 15, 1915	Do.	$S'; \frac{S'}{d}$ B.C.; 2146–2305.
	369	112	30.4	Oct. 6, 1915	Do.	$dl; \frac{S'}{d_l}$ F_2; 2217–1659.
	211	57	27.1	Nov. 18, 1915	Do.	$dl; \frac{S'}{d_l}$ B.C.; 2460.
	1,027	271	26.4	Aug. 24, 1916	Do.	$S'; \frac{S' \quad pr}{S_k \, d}$ B.C.; 4999–5110.
	3,472	949	27.3			
Star black...	1,352	522	38.6	Jan. 1, 1915	Bridges	$p_z; \frac{S'}{b \; p_z}$ B.C.; 1921–'24.
	496	203	40.9	Oct. 23, 1915	Do.	$f_r; \frac{S'}{b \; f_r}$ B.C.; 2282–'84.
	865	315	36.4	Oct. 26, 1915	Do.	$v_g m; \frac{S'}{b \; v_g m}$ B.C.; table 123, this paper.
	690	266	38.6	Dec. 22, 1915	Do.	$d_l; \frac{S' \; d_l}{b}$ B.C.; 2679–7085.
	13,104	4,944	37.7	Dec. 5, 1916	Plough.	J. E. Z., '17, p. 147; temperature; $\frac{S'}{bc}$ B.C.; table 7 ($22°$), 8_8($17°$), 8_8($22°$), 11_1($22°$), 17_2.
	16,507	6,250	37.9			
Star trefoil..	154	65	42.2	Aug. —, 1917	Morgan	
Star apterous	205	88	42.8	Nov. 18, 1916	Bridges	$a_p; \frac{S'}{a_p}$ F_2; p. 239, this paper.
Star purple..	6,766	3,010	44.5	July, 11, 1915	Do.	$S'; \frac{S'}{p_r \; c \; s_p}$ B.C., 1sts; 1836–1894
	1,027	413	40.2	Aug. 24, 1916	Do.	$S'; \frac{S' \quad pr}{S_k d}$ B.C.; 4999–5110.
	362	138	38.1	July 21, 1917	Do.	$S'; \frac{S}{p_r}$ B.C., construction; 7391, 7392.
	8,155	3,561	43.7			

TABLE 122.—*Summary of all cross-over data involving star*—continued.

Loci.	Total.	Cross-overs.	Per cent.	Date.	By—	Reference.
Star vestigial	450	195	43.3	Nov. 17, 1915	Bridges	$v_g st$; $\frac{S'}{b\ v_g st}$ B.C.; table 123, this paper.
Star curved.	6,766	3,164	46.8	July 11, 1915	Do.	S'; $\frac{S'}{pr\ c\ sp}$ B.C., 1sts; 1836–1894.
	13,104	5,959	44.4	Dec. 15, 1915	Plough	J. E. Z.; '17, p. 147; temperatures; $\frac{S'}{bc}$ B.C.; tables 7 ($12°$), 8_3($12°$), 8_5($12°$), 11_1 ($12°$), 17_3.
	19,870	9,123	46.9			
Star telescope	531	236	44.4	May 21, 1916	Bridges	t_s; $\frac{S'}{t_s}$ B.C.; 4632–'35.
Star plexus..	1,352	632	46.7	July 20, 1915	Do.	px; $\frac{S'}{b\ p_x}$ B.C.; 1921–'24.
Star fringed.	496	274	55.2	Oct. 23, 1915	Do.	fr; $\frac{S'}{b\ f_r}$ B.C.; 2282–'84.
Star pinkish.	175	74	42.3	Sept. 23, 1916	Do.	*Pinkish*; $\frac{S'}{pinkish}$ B.C.; 5267.
Star speck...	369	184	49.9	June 28, 1915	Do.	S'; $S'\ s_p$ B.C.; 1806–'07.
	6,766	3,264	48.3	July 11, 1915	Do.	S'; $\frac{S'}{pr\ c\ s_p}$ firsts; 1836–1894.
	7,135	3,448	48.3			

necting-link between that body and the other relatively poorly established loci of the experiment.

There was on hand a complex stock containing the two genes—streak and dachs (provided by Muller)—and this was used in the P_1 mating to star purple, which was made for that purpose. The F_1 star streak females were back-crossed to males of a stock of dachs purple which was brought to production simultaneously with the F_1 cultures (table 121).

In making the classification of the flies produced by the quadruple back-cross $\frac{S'}{S_t\ d}\ \frac{pr}{}$ the first separation performed was that of streak, disregarding the other characters, and including as streak only those quite obviously streak. These precautions prevented the classification of any not-streak flies among the streak, and established a uniform standard for streak among all the various classes.

The separations were of varying degrees of difficulty in the different cultures. The hardest were 4999 and 5040, which were fully as difficult as in the abandoned streak dachs back-cross. On the other hand, the separations in culture 5110 were perfectly sharp and complete.

The streak flies totaled 396, and the calculation of the cross-over values on the basis of these flies gave a star streak value of 15.9 units, a streak dachs value of 16.2, and a streak purple value of 28.8 (table 121).

From the first two of these values it appears that streak is very nearly midway between star and dachs, and that star is about 32.1 units to the left of dachs, which agrees well with the position as calculated from the star dachs data, with allowance for double crossing-over. The coincidence for star streak dachs was only 9.8, which is very low indeed. This means that double-crossing over in the left end of the chromosome, as we had earlier found to be the case in the right end, is very much lower than it is in the middle of the chromosome. This would seem to be connected in some way with the fact of median attachment of the spindle fiber, and further analysis of the problem should throw much light on synapsis and crossing-over.

The dachs purple cross-over value of 19.1 is lower than expected, and leads to the mapping of dachs somewhat nearer to black than formerly.

The calculation of the other linkage values—those of which streak is not one of the loci concerned—can be made on the total number of flies (1,027), since these values are established by the classification of fully separable characters.

A summary of all available linkage data involving star is given in table 122.

VALUATION OF STAR.

Star is now the most used second-chromosome mutant. Its viability (heterozygote) is on a par with that of the wild fly. Its position is ideal. It interferes with the classification of only one other second-chromosome character—morula—which loses in usefulness because of this conflict with star. The separability of star from the wild-type is not as clear and sharp as desirable. There is danger of overlooking some of the star flies among the wild-type. This difficulty is not general, but is much more pronounced in certain cultures, wherefore it seems likely that most of the suppression is due to modifiers. Two modifiers that markedly increase the separability of star are known, one of which is in the third chromosome (the stock of the double form being known as S^2) and the other of which is sex-linked (S^3 stock).

There is another danger in the use of star that must be constantly guarded against. Mutations of the moruloid type are very frequent and confusion has resulted from the presence of such forms in experiments supposed to contain only star. When the presence of such a mimic is once recognized steps can be taken to eliminate it and thereby remove the difficulty. One such, "pitted," apparently arose in the star dichæte stock, and through the extensive use of this stock became spread widely through our experiments and other stocks derived from these experiments. Star can therefore be used successfully only by those thoroughly familiar with it and under favorable conditions of illumination and magnification, which fact prevents its general use by students.

The double dominant form, star dichæte (dichæte being the most important third-chromosome mutant), is probably the most useful single stock we employ. For example, it has almost entirely supplanted the former methods of testing the chromosome group of a new mutant, and likewise furnishes the first test applied in determining loci within the chromosome.

NICK (v_g^n).

(Text-figure 84.)

ORIGIN OF NICK.

In an experiment to determine the cause of the linkage disturbance found in lethal 2 (Morgan and Bridges, 1916, p. 51), Bridges found a single male which showed a very slight nick or notch at the tip of the wing (culture 2012, August 7, 1915). This nick character would not have been noticed had not the fly been very exceptional in another regard, for he was otherwise wild-type (though noted as very dark), which is a condition so rare in the particular experiment that only one other wild-type male occurred in some thousands of offspring. It is our custom to test flies whose occurrence is rare in order to be sure that they are actually as they appear to be, and are not the result of error in classification or parentage. For this reason the male was out-crossed to an eosin tan vermilion female. In F_1 all the daughters were wild-type, which showed that no error had been made in classifying the fly as not-tan, and tan was the only character in the parent experiment in which there was any such difficulty in classification. The sons were eosin tan vermiliom, as expected. An F_1 pair gave in F_2 45 not-nick and 12 nick offspring (culture 2210, August 28, 1914). The nicks were equally distributed among females and males, wherefore it was known that the character was not sex-linked. The significant feature of this F_2 was that most of the nick flies showed a dark body-color like black, and there were a few other blacks that were not-nick. To test whether this body-color were really black, a "black" nick male was out-crossed to a black female from stock. All of the F_1 flies were black. The presence of black in the F_2 effectively disposed of the question of the classification of the rare fly in the lethal experiment—he was due to contamination by some method and had no place in the experiment.

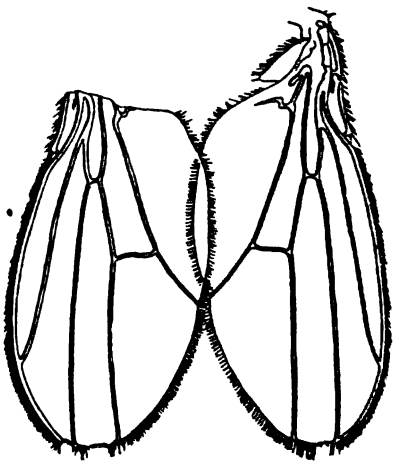
DESCRIPTION OF NICK.

Some further tests were undertaken with the character nick, since it seemed to be a hitherto unknown mutant. The main characteristic of nick is the excision of a piece of the wing-blade from the region in which the fourth longitudinal vein meets the margin—that is, at the tip and inner margin. This section may be very tiny (fig. 84) or more extensive than in an extreme "notch." The more extreme nicks

appear much like the various types of strap, except that the wings do not diverge. There may also be other excisions at the outer edge of the tip or along either margin, rarely in the outer margin, often in the inner.

CHROMOSOME CARRYING NICK.

One of the black nick males was out-crossed to a wild female and two F_2 pair cultures raised. One of these (2190) repeated the result of the first F_2, but the other (2191) gave no nick whatever. In place of nick there was present vestigial. Some of these black vestigial flies were crossed to black vestigial flies of stock and gave only black vestigial F_1 offspring. Others mated together likewise gave only black vestigial offspring. The original male must, then, have carried both black and vestigial in one of its second chromosomes.



TEXT-FIGURE 84.—Vestigial-nick compound showing a slight development of the nick. More extreme forms are scarcely to be distinguished superficially from "short" notches or from broad straps.

The fact that most of the F_2 nick flies were also black seemed to show that black and nick were in the same chromosome. But that this black-bearing chromosome was not the one carrying vestigial seemed no less clear from the fact that no vestigials had appeared in the F_2 with the black. This reasoning leads to the supposition that the original male, noted as dark, was really homozygous black, which is not impossible, provided the weakness of the color were due to age or that the male had come from a crowded or poorly fed culture.

LOCUS OF NICK.

The character nick had shown a decided linkage with black, wherefore its gene was known to be in the second chromosome. To determine its locus a back-cross was started by mating a black nick male to a star female and testing the F_1 star females by black nick males (table 123). Two of the back-cross cultures (2327, 2329) gave nick; but instead of the nick being 50 per cent of the flies as expected from a back-cross, it was only 24.2 per cent. Correspondingly there was a superabundance of black not-nick flies, so that some condition for the development of the nick character was absent from many of the flies. A calculation based on the nick flies showed that the apparent locus of nick was to the right of black and 19 units distant. This was close to the locus of vestigial and suggested that there might be some relation

between nick and vestigial. The possibility of this connection was strengthened by the fact that one other back-cross culture (2328) had given black nick in about the same frequency as had the other two, but the remaining blacks were here vestigial instead of simply black.

TABLE 123.—P_1, *black nick* ♂ × *star* ♀; *B.C.*, F_1 *star female* × *black nick* (*black vestigial* ♂ *in case of 2461.*

Oct. 26, 1915.	S' b $v_g n$		S' b $v_g n$		S' $v_g n$ b		S' b $v_g n$	
	Star.	Black nick.[1]	Star black nick.	Wild-type.	Star nick.	Black.	Star black.	Nick.
2327.....	14	7	2	8	1	7	1	..
2329.....	75	39	11	45	10	57	25	4
2422.....	31	9	8	15	1	8	16	..
2416.....	57	32; *19*	21; *18*	41	15; *7*	9	6	9; *2*
2461.....	60	*58*	*35*	32	*13*	21	9	*7*

[1] The italicised numbers refer to vestigial.

In the next generation, made from star females and black nick males (from 2329), there was one culture giving black nicks and blacks (2422), and one giving black nicks and black vestigials (2416).

VESTIGIAL-NICK COMPOUND.

It was now realized that probably not one of the nicks had failed to be heterozygous for vestigial, and it was suspected that the presence of vestigial might be a necessary condition for the development of the nick character. It was concluded that the mutant "nick" might be an allelomorph of vestigial, which by itself gave no visible difference from the wild-type (most of the many black not-nicks being homozygous for the mutant gene), but which gave the visible character "nick" when compounded with vestigial

$$\left(\frac{v_g}{v_g^n}\right)$$

The original male was by inference black vestigial in one second chromosome and black nick in the other. One of the F_1 flies had carried the black nick second chromosome and a wild second chromosome, while the other carried the black vestigial second chromosome and a wild-type second chromosome. The F_2 culture should then give 3 wild-type flies to 1 fly that would be a vestigial-nick compound and would show the nick character.

The second set of F_2 cultures gave one precisely like the first and one which gave no nick whatever, but instead gave vestigials. In this case both F_1 flies were of the type that received the black vestigial second chromosome of the black nick father.

In the back-crosses the same two kinds of F_1 flies should occur $\left(\frac{S'}{b\ \ v_g n}\right.$ and $\left.\frac{S}{b\ \ v_g}\right)$, which were both tested by back-crossing with males of the constitution $\frac{b\ \ v_g n}{b\ \ v_g}$.

TABLE 124.—P_1. *black nick* ♂ $\left(\frac{b\ \ v_g^n}{b\ \ v_g}\right)$ × *vestigial* ♂.

Nov. 17, 1915.	Vestigial.		Wild-type.		Nick.	
2464.....	162		56		85	
2465.....	99		50		56	
Total.....	261		106		141	
Feb. 3, 1916.	♀	♂	♀	♂	♀	♂
3095.....	5	1	.....	4	8	2
3096.....	31	40	5	31	35	10
3097.....	7	6	1	1	7	3
3419.....	35	30	9	15	51	15
3420.....	35	42	11	22	33	27
3421.....	42	35	14	22	48	20
Total.....	155	154	40	95	181	77

These two types of back-crosses should be in equal numbers, and 3 of the first type and 2 of the second were found.

If the above were the true explanation of the history of the nick crosses, then whenever nick is out-crossed to vestigial half of the offspring should be vestigial $\frac{v_g}{v_g}$ and half nick $\frac{v_g^n}{v_g}$. This test was applied and found to hold in part (table 124, cultures 2464 and 2465), for while half the flies were vestigial the remainder were not all nick as expected, but 141 were nick to 106 that were wild-type.

It was assumed that not all the compounds showed nick because of overlap, which might well be the case as far as the agreement with previous results went; for the nicks had quite uniformly failed to be as numerous as expected.

If the nick character is the result of a vestigial-nick compound, then it is more efficient in testing the linkage to back-cross by a black vestigial male than by a black nick male $\left(\frac{S'}{b\ \ v_g^n}\ ♀ \times bv_g ♂\right)$, for in this case twice as many nicks should appear, as though the father were himself nick. Several such tests were started, but all failed to breed except one, which happened to have come from the black vestigial second chromosome of the nick parent (culture 2461, table 123).

A stock of flies homozygous for the nick allelomorph should be obtainable by the paradoxical method of selecting against the nick as well as the vestigial flies that should appear on inbreeding the flies showing the nick character.

The lines of selected flies which show neither vestigial nor nick for some generations should be pure for the nick gene. This method was somewhat rough, but since it offered a means of carrying on the stock without extra work it was employed.

Some black nick males $\frac{b}{b}\ \frac{v_g^n}{v_g}$ were crossed to vestigial females in the course of some later experiments, and these gave the same sort of result as that noted in the first division of table 124, except that there was found to be a marked sex-limited development. While 82 per cent of the vestigial-nick female compounds showed the nick character, only about half of this percentage (45 per cent) of the males showed the nick character (table 124, last division). This sex-limitation is in the opposite direction from that previously known in the case of the vestigial-antlered compounds $\left(\frac{v_g}{v_g^a}\right)$, for there most of the compound males showed the antlered, while only a few of the females were like antlered.

DACHS-LETHAL (d_l).

ORIGIN OF DACHS-LETHAL.

The first demonstration[1] of a recessive autosomal lethal in *Drosophila* came through the effort to determine more closely the locus of star by means of its linkage relations to dachs (October 6, 1915, culture 2217).

TABLE 125.—P_1, *star* ♀ × *dachs* ♂; F_1 *star* ♀ + F_1 *star* ♂.

Sept. 12, 1915.	Star.	Dachs.	Star dachs.	Wild-type.
2141.....	30	12	3	6
2142.....	42	8	2	13
2143.....	59	11	4	10
2144.....	49	3	8	8
2145.....	15	6	..	6
2216.....	55	25	14	10
Total.....	250	65	31	53

The back-crosses which furnished the bulk of these data have already been given in the section on star; in addition some F_2 cultures from the cross of star by dachs were raised by inbreeding F_1 star males and females (table 125). It is comparatively rare that an F_2 culture is raised under such circumstances, since in general the back-cross is so much more efficient. In this case the F_2's were raised as an example of the lethal effects of homozygous star in combination with the linkage ratios. Because of the fact that all homozygous stars die, the F_2

[1] In the case of the aberrant ratios of pink, Liff (15) had suggested that an autosomal lethal might be the explanation.

ratios are simplified more than in the ordinary F_2. Thus in table 125, the classes of star dachs and wild-type are both simple cross-over classes (x), the dachs are simple non-cross-overs (n), and the star class only is complex, being a double non-cross-over plus a single cross-over ($2n+x$). In the ordinary coupling F_2, the wild-type class, which corresponds to the star of this experiment, is more complex ($3n+2x$).

Six of the F_2 cultures gave the expected results, with about 33 per cent of crossing-over between star and dachs; the other culture (2217, table 126) *gave no dachs whatever* among 116 flies, one-third of which were expected to be dachs.

TABLE 126.—*Progeny from star males and females heterozygous for dachs lethal* $\left(\frac{S' +}{+ d_l}\right)$.

Oct. 6, 1915.	Star.	Dachs.	Star dachs.	Wild-type.
2217	93	.....	.....	23
2345	68	.....	.....	11
2423	104	.....	.....	13
2592	68	.....	.....	6
2593	65	.....	.....	11
2652	42	.....	.....	6
2653	15	.....	.....	3
2658	69	.....	.....	16
2659	102	.....	.....	23
Total	626	.....	.....	112
2344	113	.....	.....	50
2346	35	.....	.....	10

DACHS-DEFICIENCY?

The phenomena of "deficiency" had just been worked out in detail in the case of forked-bar deficiency (Bridges, 1917), and accordingly the non-appearance of the dachs where both parents were expected to be heterozygous was immediately attributed to the occurrence of deficiency for the dachs gene. It was thought that the character dachs was unable to appear because of the physical absence or the complete inactivation of the dachs gene. One of the most striking features of forked-bar deficiency had been the lethal nature of the change. The same process that had removed the genes for forked and bar and the intermediate region had replaced their action with a lethal agency. Immediately the supposed case of dachs-deficiency was examined to see whether in it also the elimination of a gene had resulted in the substitution of a lethal. The ratio of 23 wild-type to 93 stars showed unmistakably that a lethal agency was operative; that the seat of

its activity was in the chromosome which had contained dachs; and further, that its locus, as judged from the linkage with star, was certainly very close to that normally occupied by dachs.

The case for dachs-deficiency was further strengthened when, as the result of tests, a third correspondence to forked-deficiency was established. A female having forked-deficiency in one X and forked in the other is in effect haploid for the forked gene, and accordingly such a female shows the forked character just as in the male, which is normally haploid for forked (and for all other sex-linked genes).

TABLE 127.—P_1, *star flies from dachs-lethal stock out-crossed to dachs.*

Nov. 17, 1915.		Star.	Dachs.	Star d'achs.	Wild-type.
2486...	$\frac{S' +}{+ d_l}$ ♂	74	28	0	0
2487...		53	58	0	0
2460....	$\frac{S' +}{+ d_l}$ ♀	88	66	16	41
2458....	$\frac{S' +}{+ +}$ ♀	62	.....	.....	71

If a fly were carrying "dachs-deficiency" in one second chromosome and star in the other and were out-crossed to dachs, then half the offspring should be dachs, since these flies should carry the dachs gene in one second chromosome and in the other no normal gene to oppose its action. When the test was made half of the offspring were dachs and half were not (table 127). In appearance these dachs flies $\left(\frac{d}{d}\right)$ were indistinguishable from the dachs flies of regular stock. The dachs flies were distributed according to the usual linkage relations of star and dachs. One pair failed to give dachs offspring (2458), corresponding to the crossing-over that occurs normally between star and dachs whereby a certain proportion of the star descendents are not heterozygous for the lethal.

BALANCED LETHALS.

It was obvious that the stock had to be carried on as a recessive autosomal lethal—that is, by mating together two flies each heterozygous for the lethal. The most advantageous method of doing this was found to be to use the flies heterozygous for star also $\left(\frac{S'}{d_l}\right)$, since in this case advantage could be taken of the fact that most of the flies which would be homozygous for not-lethal would at the same time be homozygous for star and hence be eliminated. Most of the stars in each generation would continue to be heterozygous for the lethal.

Only those which resulted from crossing-over between star and the ocus for dachs would fail to carry the lethal. If these two loci had been closer together, then fewer such cross-overs would occur and selection could be correspondingly relaxed. In the case of a pure breeding stock of "beaded," Muller found that there was an autosomal lethal in the not-beaded third chromosome, and very close indeed to the locus of the beaded allelomorph, so that no selection at all was needed. This principle, first used consciously in carrying on the stock of dachs-lethal, was called by Muller "balanced lethals" as worked out by him in the analysis of the beaded stock. Muller has shown that this principle has wide application, and may solve some of the knotty problems of the genetics of *Oenothera*, such as pure-breeding heterozygotes, the continual production of rare forms called mutants (which by this principle are due to crossing-over rather than to a fresh occurrence of the mutative process), and also the appearance of twin hybrids from certain crosses.

It was quickly recognized that the dachs-deficiency explanation was alternative to that of a simple recessive autosomal lethal occurring in a locus close to that of dachs, the recessive dachs gene being present and unchanged, but prevented from giving rise to the dachs character, because all (or nearly all) of the homozygous dachs flies were also homozygous lethal, and hence never appeared as adults. All of the three parallels to forked-deficiency were equally explainable on the linked lethal view. A possible method of distinguishing between the two conditions was offered by the appearance or non-appearance of dachs flies upon inbreeding. If the phenomena were due to dachs-deficiency, then, of course, no dachs could ever appear, since the lethal effect involved the dachs locus itself. But if two separate and distinct loci were involved—dachs and a neighboring lethal locus—then by crossing-over between them dachs should reappear. For this reason a most careful count was kept of the early stock cultures, which were run by the method of inbreeding. For four generations this was continued (table 126) and not a single dachs fly appeared among the flies. Besides the pair cultures recorded in table 126 (which were necessary in order to avoid all danger of losing the stock by crossing-over between star and the lethal), many other mass-cultures were raised for the purpose of giving full opportunity for dachs to reappear. These were not counted, since the composition of the parents was of two sorts and the ratios correspondingly confused. Approximately 5,000 flies were examined, however, without finding any dachs.

The appearance of a dachs fly would have established the linked lethal view; but the non-appearance of such flies did not prove the deficiency view, but only that if a linked lethal were present its locus was extraordinarily close to that of dachs. Such an appearance of a dachs fly would be parallel to the appearance of certain *Oenothera* "mutants," according to the application made by Muller.

There was another possible method of distinguishing between these views, which was tried. It had been found that the occurrence of the forked-bar deficiency had distributed the linkage relations in the first chromosome in a definite way. All crossing-over in the region between forked and bar was eliminated, as proved by direct tests with forked and bar, and likewise by tests of the decrease in the amount of crossing-over from that which nominally occurs between the nearest loci on either side, namely, rudimentary and fused. In the case of dachs-lethal there was no other gene known to be included in the deficient region itself, so that direct tests were impossible; and even worse, there were no loci close enough to dachs to give a measure of the decrease unless it were very marked. It was thought possible that a rather extensive disturbance might be initiated by a relatively short deficiency, since the shortened chromosome might well prevent perfect synapsis for a much longer region because of the "pucker."

The only practical but unsatisfactory test that could be made was through black, which was the nearest workable locus to the right, and star, which was the only locus to the left that could be used without inaccuracy.

If the dachs locus were deficient it could still be controlled by means of the haploid dachs flies produced by testing with dachs. Thus the proposed experiment involved a female carrying a star dachs-deficient second and a black second chromosome, to be tested by means of a dachs black male. Star and dachs were put in the same chromosome because that method was far easier, and also because the reciprocal back-cross $\left(\frac{S' \quad d_l}{b} \; ♂ \times b \; ♀\right)$ offered a means of carrying on the dachs-lethal stock with no opportunity for crossing-over, since the only heterozygote was the male, in which sex no crossing-over occurs. The stock had been run with star and dachs-lethal in opposite second chromosomes. To get them into the same chromosome a female $\left(\frac{S'}{d_l}\right)$ was out-crossed to a dachs male. The star dachs cross-over offspring contained a chromosome of the desired composition $\left(\frac{S' \quad d_l}{d}\right)$. To be sure of retaining this chromosome, and not getting a plain star dachs chromosome by crossing-over, a male of this type was used in the next step, which was an out-cross to black (culture 2613). All of the star offspring of this cross were of the desired composition $\left(\frac{S' \quad d_l}{b}\right)$. Stock was started by mating such males to black females and repeating each generation (table 128). No special pains need be taken to see that the females are virgin in this stock, which is an advantage not possessed by the similar selected stocks of the sex-linked mutants where the heterozygote has to be the female and virgin.

The females back-crossed to dachs black males produced the results shown in table 129 (cultures 2679, 2680, 2681).

The crossing-over between star and dachs was found to be normal, but the crossing-over between dachs and black was the lowest ever encountered (outside recognized linkage variations), being only 11.1 per cent, while the mean calculated from 6,725 other flies was 17.8 per

TABLE 128.—*Cultures of dachs-lethal stock,* $\frac{S'\ d_l}{b}$ ♂ × *b* ♀.

Dec. 19, 1915.	Star.	Black.
2655.....	55	48
2656.....	12	8
2657.....	135	151
2761.....	148	163
Total....	350	370

cent. This same experiment was repeated in 1917 (cultures 7083, 7085, table 129) and the same result was obtained, since the crossing-over between dachs and black was only 10.2 per cent. The lowest regular value found for dachs black was 16.7 (found by Muller in his progeny tests.)

It would seem that these values, which represent a decrease of 39 per cent from the standard values, are sufficiently aberrant to prove that the case is not to be explained by a simple lethal, linked to dachs.

TABLE 129.—P_1, *star dachs-lethal* ♀ $\left(\frac{S'\ d_l}{d}\right)$ × *black* ♂; *B.C.*, F_1 *star* ♀ $\left(\frac{S'\ d_l}{b}\right)$ × *dachs black* (♂) ♂.

Dec. 22, 1915.	$\frac{S'\ d_l}{b}$		$\frac{S'\ \ b}{d_l}$		$\frac{S'\ d_l\ \ b}{\ \ \ \ \ \ }$		$\frac{S'\ \ \ \ \ \ }{d_l\ \ b}$		Total.
	Star dachs.	Black.	Star black.	Dachs.	Star dachs black.	Wild-type.	Star.	Dachs black.	
2679.....	37	29	13	16	5	9	.....	.....	109
2680.....	67	81	36	28	8	15	1	.....	236
2681.....	29	48	16	21	7	7	1	.....	129
7083.....	34	52	20	28	5	8	.....	1	148
7085.....	20	22	12	6	4	2	1	1	68
Total.....	187	232	97	99	29	41	3	2	690

There is a possible escape from the conclusion that the case is one of dachs-deficiency, through the additional assumption of a "cross-over gene" which will give this specific disturbance of crossing-over.

Sturtevant has found two, and Bridges one such mutation in the second chromosome; but none of these gives a result very closely com-

parable to that found here. It is, however, possible that the one found by Bridges (C IIs) may on further investigation be found comparable.

On the whole, the evidence is in entire agreement with the assumption of dachs-deficiency, but at the same time is not in such disagreement with the linked-lethal view as to disprove it. Before either alternative can be dropped it is necessary to repeat the two tests which offer definite solution—the search for dachs flies from an inbred line and far more rigid tests of the system of linkage that is present. Meanwhile, the ambiguous term "dachs-lethal" will be retained to cover both possibilities..

SQUAT (S_q).

(Text-fig. 85.)

ORIGIN OF SQUAT.

In tracing the course of "high" non-disjunction (Bridges, 1916) a female heterozygous for the sex-linked genes vermilion, sable, garnet, and forked $\left(\frac{v\ +\ +\ f}{+\ s\ g\ +}\right)$ was tested for the occurrence and percentage of secondary exceptions by out-crossing to a male from the bar (sex-linked dominant) stock. One of the regular vermilion forked sons of this pair (culture No. 2480) was found (November 29, 1915) whose wings, legs, and body were considerably shorter than normal, giving a "squat" appearance to the fly. Only one such squat fly was found among 372 offspring from this pair, which in such cases is always a strong indication that the mutant is either dominant or sex-linked.

In order that a non-sex-linked recessive should appear in a culture, both parents must be heterozygous for the gene, and in such cases the recessive character appears as a quarter of the individuals and not as a single individual, as was here observed.

INHERITANCE OF SQUAT.

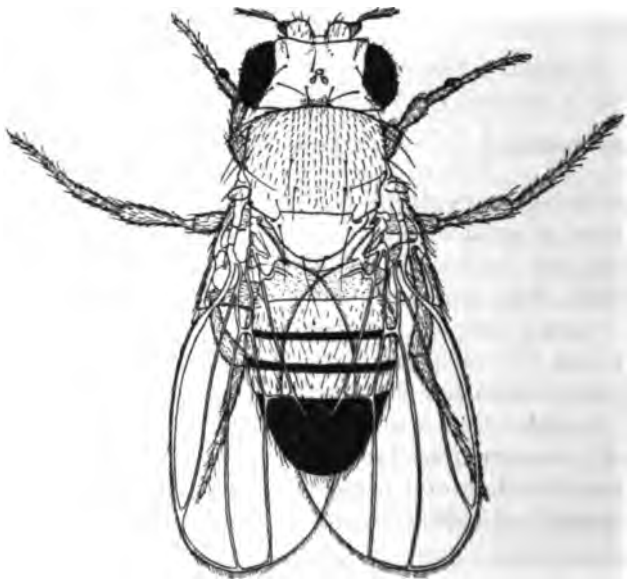
The squat male was out-crossed to a wild female, and in F_1 produced (culture 2635) a total of 53 squat flies to 47 not-squats. This means that the mutant is a dominant and the original male was likewise a heterozygous dominant. That it is an autosomal dominant, rather than a sex-linked dominant, was proved by the fact that half (22) of the F_1 squats were males; had the squat been sex-linked none of the sons could have shown the mutant, since their single X chromosome comes from their mother and not from their father.

A squat male and female from F_1 were inbred, and gave in F_2 121 squats to 53 not-squats (No. 2728). This seemed to be an approximation of a 3 : 1 ratio rather than of a 2 : 1 ratio, and was thought to indicate that the dominant is probably not lethal when homozygous.

Nearly all of our other dominant autosomal mutants are lethal when homozygous, and therefore give 2 : 1 ratios when inbred, as in the type case of the yellow mouse.

DESCRIPTION OF SQUAT.

The squat flies seen in culture 2636, and in subsequent cultures, may be more exactly described by aid of the drawing of the somewhat atypical specimen of figure 85. The most striking change, and the one most dependable in classification, is that of the wing, which is about 80 per cent the normal length, is slightly broader than normal, and has a blunt end. The whole wing is of a somewhat weaker texture, with a tendency to droop like "arc." The color of the wing is somewhat cloudy and brownish instead of the clear gray of the normal. The wings are also sometimes slightly divergent. The thorax is short, broad, and rather flattened on top. The head likewise is broad from side to side, and quite often the eye has a protruding lump which is caused by an extra antenna pushing partly or entirely through. The legs are weak and shortened, especially in the basal joints. None of the above changes are very marked or of uniform occurrence. One learns to recognize the type much as in the case of certain wild species, or *Oenothera* mutants, by the ensemble of slight differences.



TEXT-FIGURE 85.—Squat.

CHROMOSOME OF SQUAT.

To test whether squat is third-chromosome or not, squat males were out-crossed to the third-chromosome dominant dichæte. Both F_1 pairs (2730, 2829) gave very few squats, and these difficult of classification. It seemed, and this has proven to be the case, that the squat character behaves as does truncate and several other of our mutations. Presumably, like truncate, it owes most of this evanescence to its extreme sensitiveness to modification, both in intensification and suppression, brought about by the action of different combinations of

genes. Such eclipses are only temporary, but are serious in that they spoil the usefulness of the mutation as a working tool.

One of the F_1 squat dichæte males was out-crossed to a wild female and produced four classes of offspring (culture 2993; S_s 21 : D′ 20 : S_s D′ 20 : +24). Since this was a back-cross test of the male, it is evident that squat is not in the third chromosome. If squat were third-chromosome there would have been no squat dichætes.

At the time that the dichæte test was made a parallel cross to star was started to test the relation of squat to the second chromosome. Here also in the F_1 cultures (2738, 2828) the squat could be distinguished only poorly.

The back-cross test of the male attempted in this case failed, probably through sterility. But the answer was obtained, though less surely, by a female test started at the same time. One of the F_1 star squat females $\left(\frac{S'}{S_s}\right)$, when out-crossed to a wild male gave among the offspring a few star squats (culture 2827). Since the squat was poor, no accurate records were kept, though it seemed very probable that squat was showing linkage to star. That the eclipse of squat did not mean extinction was proved in the next generation; for a star female and a not-star male, both selected as showing no squat, produced squat offspring (3061). These squats were dominants, not recessives, as proved by out-crossing them to wild flies, whereupon in F_1 nearly half of the flies were squats (3339).

OTHER MUTATIONS.

In culture 3061, just described, the second-chromosome recessive, "narrow" wings was found (February 7, 1916). From the linkage with star which it showed, it had evidently been introduced to the cross through star.

In culture 3858, which was simply a stock culture of squats whose parents were taken from 3061, there reappeared the second-chromosome recessive mutant "commas," which had apparently been carrried along in the same chromosome with squat, but being recessive had hitherto no opportunity to show itself.

LOCUS OF SQUAT.

It had by now become apparent that squat was second-chromosome, and it was thought advisable to make a rough determination of its locus. Squat was therefore crossed to black plexus and a back-cross test of the female made. Black is a control for the middle and plexus for the right end of the second chromosome. No control of the left end was made, since the fairly free crossing-over between star and squat observed in some stock cultures had made it evident that the locus of squat is fairly distant from the left end of the chromosome.

The one back-cross culture that produced results (4044, table 130) showed that squat is not far from black, there being 9 cross-overs in a total of 82 flies, or 11.0 per cent.

The pair of complementary classes with the smallest sum is squat black and plexus, which are therefore probably the double cross-overs. If this is true, then squat lies to the left of black, a position which agrees with the amount of its crossing-over with star and also with the high value (47.6) for squat-plexus.

TABLE 130.—P_1, *squat* ♀ × *black plexus* ♂; *B.C.*, F_1 *squat* ♀ $\left(\frac{S_q}{b\ p_x}\right)$ *back crossed by black plexus* ♂.

Apr. 3, 1916.	S_q / p_x	b	S_q / p_x	+	S_q / p_x	b	S_q / b	p_x	Total.
No. 4044.....	15	24	3	2	13	21	3	1	82

Because of the small number of flies, this determination is not very accurate, but since the character is so poor it was not thought best to do any more then, and the problem may never be taken up again.

A stock of squat was made up and has since been running without selection. A recent (February 1918) examination of the stock showed only an occasional squat, one of which was drawn (figure 85). The original stock was not pure and the present scarcity of squats may be due to their poor viability in competition with the non-squat sibs, to a possible lethal nature of the squat homozygote, and to some extent to "eclipse" of the squat character.

LETHAL II*a* SAME AS (l_{IIa}).

ORIGIN OF LETHAL II*a*.

In looking over the star black curved stock (December 4, 1915) in search of a virgin black curved female, Bridges noticed that one of the black curved males had an eye-color much like purple. This male was out-crossed to a wild female, and several pairs of the wild-type F_1 flies were bred for the F_2 generation. It was suspected that the eye-color, called crimson, was sex-linked, and this was confirmed by the fact that crimson reappeared only in the F_2 males, where it constituted about half the flies. As soon as the sex-linkage of crimson was established the counts on the F_2 cultures were stopped and the cultures were thrown away in favor of a back-cross culture, which had been made by mating the original crimson male to one of his wild-type daughters. This back-cross culture was continued, since it gave crimson females as well as crimson males and a stock was directly obtainable. As a side issue it was decided to make counts on this back-cross culture to illustrate the independence of the new mutant crimson and the second-chromosome characters. In making the counts only crimson and

curved were classified and no attention was paid to black. The result was rather unexpected, for while crimson and curved proved to be independent (56 per cent recombinations), the curved flies constituted only 31.4 per cent of the flies instead of 50.0 per cent (table 131). This difference was not clearly recognized until the counts were totaled,

TABLE 131.—*P_1, crimson black curved ♂ × wild ♀; B. C., F_1 wild-type ♀ + crimson black curved father.*

Dec. 21, 1915.	Wild-type.	Crimson.	Curved.	Crimson curved.
2675.....	49	69	27	27

and there were then no more flies hatching. However, one of these F_2 cultures was recovered from the discards and a count was taken of it. This count showed no black curved flies whatever, and far too few blacks and curved. The case of dachs-lethal was then being followed, so that a hypothesis was known that covered this situation. It was concluded that an autosomal lethal had arisen by mutation in this second chromosome at a locus between black and curved. No black curved flies

TABLE 132.—*Offspring given by pairs of wild-type flies from an F_2 (F_3) parallel to the back-cross of table 131.*

Jan. 15, 1916.	Wild-type.	Black.	Curved.	Black curved.
2840.....	231	8	8	1
2863.....	86	2	5	.....
Total.....	317	10	13	1
2861.....	91	.....	.....	.....
2864.....	234	.....	.....	.....
Total.....	335	.....	.....	.....
2859.....	110	42	6	.....
2860.....	70	.....	5	.....
2862.....	39	.....	4	5

appeared in the F_2, because every black curved zygote (except the rare double cross-over) was at the same time homozygous for the lethal. By crossing-over between black and the lethal $\left(\frac{b\ l\ c}{+\ +\ +} \longrightarrow \frac{b\ |\ +\ +}{+\ |\ l\ c}\right)$ a few black flies would be produced $\left(\frac{b\ +\ +}{b\ l\ c}\right)$; likewise the few curved flies corresponded to crossing-over between the lethal and curved.

If this were the explanation, then most of the wild-type flies should be of the same constitution as the F_1 flies $\left(\frac{b\ l\ c}{+\ +\ +}\right)$ and when mated together should repeat the F_2 result.

Seven pairs of F_2 (or more likely F_3) wild-type flies gave offspring (table 132). Of these pairs, two (2840, 2863) proved to have both parents of the original constitution $\left(\frac{b\ l\ c}{+\ +\ +}\right)$. Two others (2861, 2864) produced only small wild-type offspring, from which it was evident that at least one parent of each had carried neither black nor curved, and had come from the non-crossover chromosome which was alternative to the b l c chromosome. The remaining three pairs contained various cross-over chromosomes. Thus, 2859 came from

$$\frac{b\ +\ +}{+\ l\ c}\ ♀ + \frac{b\ l\ c}{+\ +\ +}\ ♂,$$

2860 from $\frac{+\ l\ c}{+\ +\ +}\ ♂ + \frac{b\ l\ c}{+\ +\ +}\ ♀$, 2862 from $\frac{b + c}{+\ +\ +}\ ♀ + \frac{b\ l\ c}{+\ +\ +}\ ♂$.

The mother of 2859 contained two cross-over chromosomes $\left(\frac{b\ +\ +}{+\ l\ c}\right)$ and must therefore have been an F_3 individual, as more were expected to be.

LOCUS OF LETHAL II*a*.

The composition of these flies is important, since from their offspring calculations of the amounts of crossing-over between the lethal and both black and curved were made. The first culture in which the lethal appeared (2675, table 131) was of the type most advantageous for the study of the crossing-over relations because of being in the form of a back-cross for black and curved. Unfortunately, no counts had been taken on black, so that only the lethal curved value could be calculated. Both the curved and the not-curved classes produced by this cross are composite, the curved class being composed of two cross-overs and a non-crossover class ($2x+n$), while conversely the not-curved class is $2n+x$. The solution of the equations

$$2x+n=54 \qquad x+2n=118$$

gives $x=-3.4$ and $n=60.7$. The fact that x has a minus sign is easily accounted for by probable error, and only means that the loci of the lethal and curved are close enough together so that a small deviation of the classes gives an apparently impossible cross-over value.

In cultures 2840 (and 2863) the composition of the not-curved class is $3n+2x$ and of the curved class simply x. The not-curved flies totaled 327 and the curved 14, from which $x=14$ and $n=99.7$. The lethal curved cross-over value is thus 12.3. Likewise the black lethal cross-over value is 9.8. Culture 2859 furnishes data on the lethal curved value according to the equations

$$3n+2x=152 \qquad x=6$$

from which the cross-over value is 11.4, comparable with the 12.3 just found from 2840+2863. The condition in 2859 with respect to black

and lethal is the reverse of that from lethal and curved, since black and the lethal are in different homologues. The equations in this case are

$$2n+3x\times 116 \qquad n=42$$

from which the black lethal value is 20.3.

Culture 2860 gave $x=5$, $n=20$ and a lethal curved cross-over value of 20.

The foregoing cultures have given in the case of black and lethal a total value of n of 144.7 and of x of 21.7, so that the cross-over value is 13.0. Likewise for lethal-curved, $n=227.1$, $x=21.6$, and the cross-over value is 8.7.

STOCK OF LETHAL II*a*.

It was foreseen that stock of this lethal, which was called lethal IIa (the "II" designating the chromosome, and the "a" denoting the first of this series), could not be run advantageously by means of cultures such as were made from the wild-type flies of the original F_2.

A stock could be maintained by taking advantage of the fact of no crossing-over in the male, if males carrying the lethal in one second chromosome and some recessive in the other were back-crossed in each generation to females homozygous for this same recessive. An efficient scheme for obtaining such a stock was devised as follows: The original F_2 flies appeared only in the three classes: wild-type, black, and curved. The constitution of any given wild-type fly could not be told by inspection, but the case was different with the blacks and curveds. Their father had carried black, lethal, and curved all in the same second chromosome, and since there is no crossing-over in a male, every sperm which carried one of these genes carried all. Therefore every black fly in the F_2 should have the black lethal curved second chromosome of the father.

The maternal second chromosome is in this case a cross-over chromosome, carrying black not-lethal, and not-curved. If such a male $\left(\frac{b\ +\ +}{b\ l\ c}\right)$, were crossed to a curved female, the offspring should be of two kinds—wild-type $\left(\frac{b\ +\ +}{+\ +\ c}\right)$ and curved $\left(\frac{b\ l\ c}{+\ +\ c}\right)$, and every one of these curved flies should carry the lethal in the manner required. Instead of simple curved, a black purple curved plexus speck ($\pi-$) female was used in this out-cross—the object being to combine the $\pi-$ stock with the l_{IIa} stock so that no separate $\pi-$ or l_{IIa} stocks need be maintained. Three such out-crosses of F_2 black males to $\pi-$ females were made. Two of these gave only black offspring (culture 2865, 155 blacks; culture 2866, 182 blacks), and no black curved, which showed that the males had been cross-overs belonging to the F_3 generation. To check up this analysis, five F_2 cultures were raised from one of them

(2866), and, as expected, gave no lethal result (table 133, not separated for plexus).

The third culture, 2867, gave 40 black curved and 41 black flies and seemed to be the type to be used. Accordingly several of the black curved males, supposed to be of the constitution $\frac{\pi -}{b\ l\ c}$ were out-crossed to $\pi -$ females to give the required stable stock. It was well that three F_2 cultures were raised at the same time, for these test cultures

TABLE 133.—*P_1, black ♂ × black purple curved plexus speck ♀; F_1 black ♀ + F_1 black ♂.*

Feb. 8, 1916.	Black purple curved speck.	Black.	Black purple.	Black curved speck.	Black purple curved.	Black speck.	Black purple speck.	Black curved.
3203.....	24	109	4	13	11	20	2	5
3204.....	23	214	4	8	22	16	.....	.....
3206.....	27	167	9	11	21	23	.....	.....
3207.....	28	171	10	15	15	15	1	5
3208.....	38	181	21	16	23	28	.....	3
Total.....	140	842	48	63	98	98	3	13

(table 134) proved that the male was not carrying lethal and had in fact been an F_2 double cross-over of the constitution $\frac{b + c}{+ + +}$.

After this failure to secure stock from flies of the original F_2, the attempt was repeated successfully with black flies of the derived culture (F_2) which had the same constitution as the original F_2, but in which flies of the succeeding overlapping generations were known not

TABLE 134.—*P_1, black ♂ × black purple curved plexus speck ♀; F_1 black curved ♀ + F_1 black curved ♂.*

Feb. 7, 1916.	Black purple curved speck.	Black curved.	Black purple curved.	Black curved speck.
3168.....	34	124	18	14
3169.....	33	123	21	23
3201.....	15	73	9	8
Total.....	82	320	48	45

to be present. Two such black males by $\pi -$ females gave as expected black and black curved offspring (3112: $b = 81, b\ c = 67$; 3197: $b = 67$, $b\ c = 53$). A stock from this source was turned into the stock-room after tests had shown that it was carrying the lethal. The tests (table 135) consisted of inbreeding some of the black curved males and females, which proved to have the required constitution $\left(\frac{b\ p_r + c\ p_s\ s_p}{b + l_{II}\ c + +}\right)$.

These tests brought out the interesting point that in crosses in which a recessive and a lethal are in opposite chromosomes $\begin{pmatrix} r & + \\ + & l \end{pmatrix}$, the percentage of the recessives in F_2 is a constant value 33.3, irrespective of how much or how little crossing-over there is between the two loci. Thus, the recessives purple, plexus, and speck are at very different distances from the locus of the lethal, yet the percentages of appearance of all these is practically the same ($p_r = 37.0$, $p_x = 34.0$, $s_p = 35.3$, though slightly higher than the expected value of 33.3.

TABLE 135.—*The offspring from pairs of black curved flies from* l_{IIa} *stock* $\left(\frac{b\, p_r + c\, p_x\, s_p}{b + l_{IIa} + +}\right)$.

[All flies were black curved and showed also the subdivisions in the table.]

Feb. 29, 1916.	Purple plexus speck.	"Wild-type."	Purple.	Plexus speck.	Purple plexus.	Speck.	Purple speck.	Plexus.
3535.....	21	53	18	18	3	3	.....	2
3551.....	17	54	15	15	1	1	4	1
3552.....	24	65	22	18	1	3	2	.....
3553.....	20	46	21	19	3	2	2	.....
Total.....	82	218	76	67	8	9	8	3

TELESCOPE (t_s).

(Plate 7, figure 6.)

ORIGIN OF TELESCOPE.

In determining the locus of the sex-linked mutant "crooked bristles" (locus 38.0, allelomorph of furrowed) several back-cross tests were made of females carrying vermilion, crooked, sable, and garnet in one X, and only wild-type genes in the other. One of these tests gave slightly less than a quarter of the flies with "telescope" abdomens (2735, December 27, 1915).

DESCRIPTION OF TELESCOPE.

The abdomens of the "telescope" flies tend to retain the drawn-out appearance that freshly hatched flies have. The segments are slightly separated from one another instead of overlapping. The pigmentation and chitinization of the abdomen remain weak and there is a wet (glazed) appearance to the entire surface of the body. The wings droop at the sides and diverge, this character being very useful for identification. Each band is sunken at the middle, with slightly raised edges.

INHERITANCE OF TELESCOPE.

That this character was not sex-linked was seen at once, since in the culture in which it was first found it appeared in females as well as males and showed no linkage to any of the four sex-linked characters

present. A mass-culture of the telescope females and telescope males which showed none of the sex-linked character was made (2867). This culture failed, probably because of sterility rather than from cultural conditions, since some of these same flies remated to purple, in order to start a second-chromosome linkage test with telescope failed to produce offspring (3120, 3121, 3122). A second mass-culture of telescope (2906) produced a very few flies, from which a successful outcross of a telescope female to a male from the pink spineless stock (third-chromosome recessive) was made (3213; 3214 sterile). Several F_1 pairs were started, of which one (3503) produced offspring. These offspring represented a 9 : 3 : 3 : 1 ratio of telescope and pink (+ 188, *t.* 61, *p* 44, *t.p* 12; disregarding spineless), which proved that telescope was not in the third chromosome.

This culture furnished (March 4, 1916) one of the most valuable autosomal characters, hairless, a third-chromosome dominant which is fully viable (though lethal when homozygous), which is easy of classification, which does not mask any other third-chromosome character, and whose locus is advantageous.

After the discovery that telescope was not in the third chromosome it was thought certain that it was in the second, so experiments were planned on that basis. By means of the dominant "star," at least a rough approximation of the locus was possible. Accordingly several out-crosses to star were made en masse (3848, 3849, 3854), of which 3854 alone produced offspring. In view of the sterility so far encountered it was thought best not to attempt back-crosses, but to raise F_2 which involves the mating only of not-telescope flies. The first tests were to check up the assumption that telescope was second-chromosome by means of a male test. This was done by pairing the F_1 star males $\left(\frac{S' +}{+ \; t_e}\right)$ and F_1 wild-type females $\left(\frac{+}{t_e}\right)$. The telescope offspring, then, constitute a back-cross test. The result proved, as expected, that the telescope is second-chromosome, for none of the back-cross telescopes were star (table 136).

TABLE 136.—*P_1, telescope ♀ ♀ × star ♂ ♂; F_1 star ♂ + F_1 wild-type ♀.*

Apr. 7, 1916.	Star.	Wild-type.	Telescope.	Star telescope.
4098.....	167	55	29	0
4099.....	125	64	44	0
Total.....	292	119	73	0

At this time the mass stocks of telescope were producing better (4313, 4516, 4636), so some female back-cross tests were attempted. Of the first lot one (4400) succeeded fairly well, but no counts were

made. In the next generation, however, two cultures produced abundant offspring (table 137). Crossing-over between star and telescope was very free, there being 44.4 per cent observed crossing-over in a total of 531 flies. By comparison of this value with the other star cross-over values it seems likely that telescope is to the right of black, probably to the right of purple, and most probably in the neighborhood of vestigial. A position at 66.5 units to the right of star is given by a correction of the cross-over value, according to the probable coincidence of 100.

TABLE 137.—P_1, *telescope* ♀ ♀ × *star* ♂ ♂; *B. C.*, F_1 *star* ♀ × *telescope* ♂ ♂.

May 21, 1916.	Star.	Telescope.	Star telescope.	Wild-type.
4632.....	4	6	8	3
4634.....	76	86	45	60
4635.....	58	65	54	66
Total.....	138	157	107	129

Time was then lacking for testing this location further. If the locus should be found to be to the right of curved the mutant would be valuable for some purposes, since the character is fairly easy of classification and the sterility seems less pronounced. Pending further tests, the stock was rearranged so that the danger of loss through sterility was eliminated and also the simple stocks of purple and telescope were replaced by a single stock. Telescope males (from 4634) were out-crossed to purple females of pure stock, and the F_1 wild-type males were again out-crossed to purple females. In the following generation, because of no crossing-over in the male, only two classes were produced, purple and wild-type $\left(\frac{p_r}{p_r}, \frac{p_r}{t_e}\right)$. By mating the wild-type males to the purple females, which do not need to be virgin, the stock is renewed each generation.

SECOND-CHROMOSOME "MODIFIERS" FOR DICHÆTE BRISTLE-NUMBER.

Families of dichætes that differ in mean bristle-number have been established by Sturtevant through selection (August 3, 1916). That these differences are in part due to one or more second-chromosome modifying genes has been shown by the following method:

A dichæte of a selected line was crossed to speck of a stock that had been long and closely inbred in order that it might become homozygous. Dichæte being dominant, half of the offspring were dichæte, and all of them were heterozygous for speck. The F_1 dichæte males were then back-crossed to speck females. They produced dichæte offspring that were not-speck and as many others that were speck; from their father

these two classes of offspring received second chromosomes that came from the two original stocks. If those original stocks differed in second-chromosome modifiers the two classes should differ in mean bristle-number. This has, in fact, been found to be the case (see below). But if an F_1 *female* is used, these differences should be less than in the above case, provided the modifier, or modifiers, crossed over from speck. This result has also been obtained, as will appear from table 138, which shows the excess in mean bristle-number of the not-specks over the specks among the dichæte offspring from back-crosses of the type described above. The two values in any one line are from the same combination of stocks, and are therefore available for comparison of male tests with female tests.

TABLE 138.

Test of F_1 ♂.	Test of F_1 ♀.
+.852±.067	.....
+.192±.040	+.150±.128
+.439±.132	+.088±.093
+.545±.091	+.367±.073
+.345±.113	−.054±.055
+.542±.133	−.259±.125
+.202±.081	−.532±.180

These data demonstrate the existence of one or more second-chromosome modifiers for dichæte bristle-number, and show that at least one such modifier crosses over from speck.

Further details and conclusions of this selection experiment and a general discussion of the subject are given by Sturtevant in Carnegie Institution of Washington Publication No. 264.

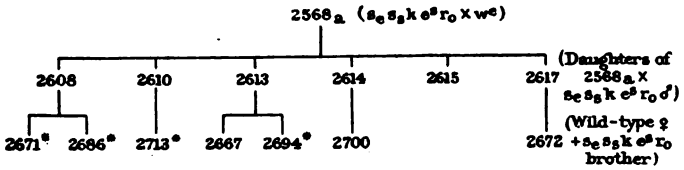
DACHSOID.

(Text-figure 86.)

ORIGIN OF DACHSOID.

In testing for the presence of a third-chromosome cross-over variation reported to be present in the eosin stock, Sturtevant out-crossed two eosin males separately to females from the stock containing the third-chromosome recessives sepia, spineless, kidney, sooty, and rough. Several of the daughters from each of these matings were back-crossed to males from the multiple recessive stock. This experiment is discussed by Sturtevant in the paper appearing herewith (Part III). From both series brother-sister pairings of the back-cross type were continued through several generations. "Dachsoid," a new mutant wing-type, appeared in four out of seven of the inbred cultures of F_2 in one strain (from 2568*a*). The first of these was observed, February 9, 1917, in

culture 2671 (A. H. S.). The relationship between the first cultures in which the character was observed is shown in the following pedigree:



The four cultures starred in the above diagram gave the following numbers of offspring:

TABLE 139.

Culture.	Not-dachsoid.	Dachsoid.	Total.
2671.....	52	7	59
2686.....	110	7	117
2694.....	184	19	203
2713.....	146	17	163
Total..	492	50	542

The viability of the dachsoid flies was evidently very poor; and the adults were so weak that all attempts to breed from them failed. For this reason the character was discarded after very little had been done with it.

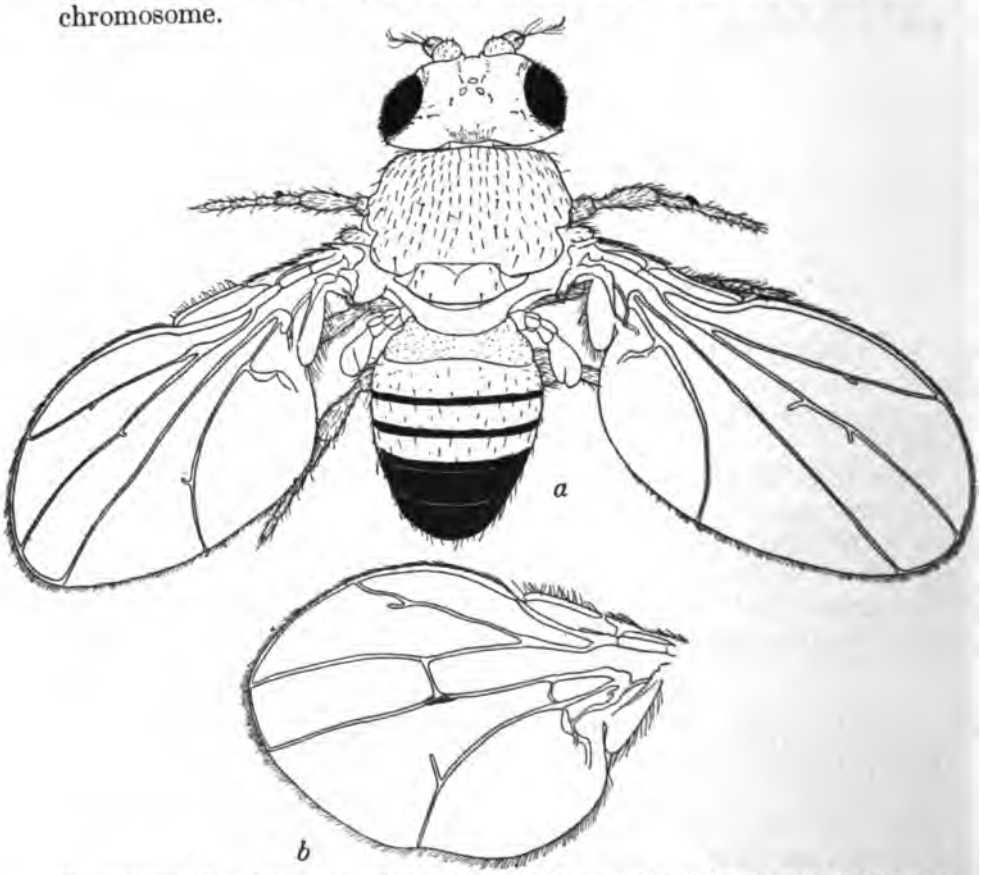
Since three daughters of the original pair produced dachsoid descendants when mated to different stock males, it is practically certain that one of the parents of 2568*a* (A. H. S.) was heterozygous for the dachsoid gene. It is not possible to determine which parent, or how long the character had been in the stock. No other experiments involving these (or any other) stocks have produced dachsoid flies.

DESCRIPTION OF DACHSOID.

As shown in figure 86, the dachsoid flies are small and all parts—head thorax, abdomen, wings, and legs—are markedly shortened, as though from pressure. The particular specimen drawn was sepia spineless kidney sooty rough, as well as dachsoid, and the short bristles and abnormal eyes are due not to the dachsoid but to spineless and kidney rough. The wings, besides being shortened, are actually broader than normal. They are held out at a wide angle from the body and have a tendency to curve. The posterior cross-vein is almost entirely gone and frequently the anterior cross-vein is similarly affected. A characteristic feature is a short branch on the second longitudinal vein similar to the remnants of the cross-veins. The hairs on the costal vein before the apex of the first vein stand out from the vein more than in wild-type flies.

CHROMOSOME CARRYING DACHSOID.

The four cultures above noted (table 139) all gave both male and female dachsoids, thus showing at once that the gene is not in the X chromosome. The mothers of all four were heterozygous for sepia, spineless, kidney, sooty, and rough—characters that cover practically the whole length of the third chromosome, and the same was true of several later cultures that also gave dachsoid. The dachsoid character was distributed quite at random with respect to these third-chromosome characters, showing that the gene is not in the third chromosome.



TEXT-FIGURE 86.—Dachsoid venation. 86*a* shows the small size of the fly, with the wing posture; 86*b* shows a typical wing.

An F_3 from a cross between speck and a fly that proved to be heterozygous for dachsoid produced 15 dachsoid, but unfortunately the speck character was not examined (2859, A. H. S.).

An F_3 pair (2926) gave a total of 40 flies, of which 4 were dachsoid, 26 speck, 10 wild-type, and none dachsoid speck. The count was aberrant in that there were far too few wild-type offspring; but the

probability is that the gene is in the second chromosome, especially in view of the evidence that it is not in the X or third chromosome.

As is suggested in its name, dachsoid has certain points of resemblance to the second-chromosome character dachs. It was therefore surmised that the gene might be allelomorphic to the dachs gene, and the following tests were made:

Six offspring of the four pairs in which dachsoid first appeared were selected at random. Two-thirds of these would be expected to be heterozygous for dachsoid; and the chance that none was heterozygous is only $\left(\frac{1}{3}\right)^6 = \frac{1}{729}$. These 6 individuals were then mated, some to homozygous dachs, some to flies heterozygous for dachs. All produced a considerable number of offspring (105 to 199 per pair) and another similar mating (to homozygous dachs) produced 9 offspring. None of the F_1 flies showed any trace of the characteristics of dachs or dachsoid, or any other unusual characters. It therefore follows that the flies heterozygous both for dachs and for dachsoid are normal in appearance. In all probability, therefore, the genes of the two characters are not allelomorphic.

THE CONSTRUCTION OF THE MAP OF THE SECOND CHROMOSOME.

The map given on page 127 is constructed on the basis of the total data available on each cross-over value. The first step taken was to collect and summarize this data in the form in which it appears in table 140.

In constructing the map of the second chromosome on the basis of all the available data, the procedure was roughly as follows: The first locus to be considered was that of black, since the "second" chromosome had originally been defined quite arbitrarily as *that chromosome which carries the gene for black and such other genes as may be found to be linked to black*, while correspondingly the third chromosome was that chromosome which carries the gene for pink and such other genes as may be found to be linked to pink. Furthermore, it so happened that of the early mutations which were stably mapped (black, purple, vestigial, and curved) black was the one located farthest to the left, and was therefore chosen as the zero-point of this early map. Even after black had been displaced from the position at zero, it still remained the base of reference of the entire second chromosome, in relation to which all other loci are mapped, either directly, in the case of those close by, or indirectly, through reference to intermediate bases in the case of those farther away. Black was therefore accepted as the constructional zero-point of the map, and all other loci were to be mapped as lying to the right or to the left of black by a specific number of units. The loci to the right, or in a

plus direction from black, were those lying on the same side of black as does curved, which was the first locus to be included with black in the second chromosome. Likewise the loci "to the left," or in a minus direction, were those lying on the opposite side of black from curved.

However, curved is too remote from black to be accurately located by direct reference to black. Accordingly, the position of these inter-

TABLE 140.—*Summary of available data on crossing-over in the second chromosome.*

Loci.	Total.	Cross-overs.	Per cent.	Loci.	Total.	Cross-overs.	Per cent.
Star streak	396	63	15.9	Black jaunty	462	1?	0.2?
Star cream b	389	86	22.1	Black purple	48,931	3,026	6.2
Star truncate	549	149	27.1	Black lethal IIa	166	22	13.0
Star black	16,507	6,250	37.9	Black vestigial	20,153	3,578	17.8
Star apterous	169	83	49.0	Black curved	62,679	14,237	22.7
Star purple	8,155	3,561	43.7	Black plexus	2,460	1,031	41.9
Star vestigial	450	195	43.3	Black fringed	496	211	42.5
Star curved	19,870	9,123	45.9	Black arc	7,592	3,237	42.6
Star trefoil	154	65	42.2	Black blistered	224	93	41.5
Star telescope	531	236	44.4	Black pinkish	736	371	51.4
Star plexus	1,352	632	46.7	Black speck	685	326	47.6
Star fringed	496	274	55.2	Black balloon	2,236	1,073	48.1
Star pinkish	175	74	42.3	Black morula	7,549	3,518	46.6
Star speck	7,135	3,448	48.3	Purple vestigial	13,601	1,609	11.8
Streak dachs	858	109	12.7	Purple curved	51,136	10,205	19.9
Streak black	462	120	26.0	Purple plexus	344	164	47.7
Streak purple	2,665	883	33.1	Purple arc	2,625	1,066	40.6
Streak vestigial	462	164	35.5	Purple speck	11,985	5,474	45.7
Streak curved	2,269	923	40.7	Purple balloon	462	218	47.2
Streak blistered	11	5	45.0	Lethal IIa curved	249	22	8.7
Streak speck	462	242	52.3	Vestigial curved	1,720	141	8.2
Streak balloon	462	242	52.3	Vestigial speck	2,054	738	35.9
Streak morula	876	405	46.3	Vestigial balloon	462	178	38.5
Dachs black	6,725	1,196	17.8	Curved speck	10,042	3,037	30.2
Dachs purple	1,489	293	19.7	Curved balloon	462	150	32.5
Dachs vestigial	5,354	1,585	29.6	Plexus speck	327	29	8.9
Dachs curved	462	145	31.4	Arc speck	2,625	156	5.9
Dachs speck	462	231	50.0	Arc morula	6,794	634	7.9
Dachs balloon	462	231	50.0	Blistered speck	36	3	8.3
Squat black	82	9	11.0	Speck balloon	462	2	0.4
Squat plexus	82	39	47.6				

mediate bases must first be mapped. Of these, purple was the first to be considered as being the closest to black, and also because its position with relation to black has been the subject of more investigation, and is more accurately determined than any other second-chromosome distance. The black purple cross-over value of 6.2 is based on 48,931 flies, and since there is certainly no double crossing-over within this distance, the value can be accepted without correction, and purple can be mapped at a locus 6.2 units to the right of black.

The third locus to be considered is vestigial, and there are open two sets of data by means of which the position of vestigial can be mapped: (1) the position of vestigial can be located directly by the purple

vestigial cross-over value of 11.8; that is, vestigial is 11.8 units to the right of purple or at 18.0 to the right of black. This location is based on a total of 13,601 flies for the purple vestigial cross-over value; (2) but there are available 20,153 flies giving a black vestigial cross-over value of 17.8. This distance is long enough so that double crossing-over occurs within it, and the cross-over value must therefore be corrected. The black purple vestigial and other back-cross experiments by which the amount of this double crossing-over has been measured show that the amount of such double crossing-over is relatively very high, the probable coincidence being about 60. The corrected value corresponding to a given coincidence and an observed cross-over value can be calculated closely enough for our present purposes by aid of the following equation:

$$x^2 - \frac{x}{C} = -\frac{o}{2C}$$

in which C = the given coincidence, o = the observed cross-over value, and $2x$ = the corrected distance, all expressed as decimal fractions rather than as percentages. The correction corresponding to a coincidence of 60 amounts to about 1.1 units on an observed value of 17.8, so that the locus of vestigial is 18.9 units to the right of black on the basis of the black vestigial data. However, the precision of the location based on values that must be corrected decreases rapidly with the increase in the size and consequent uncertainty of the correction. Although the position of vestigial at 18.9 is based on 20,168 flies, the value of each fly represented in the black vestigial cross-over data is not as great as the value of each fly in the purple vestigial experiments, where the coincidence is probably zero and no correction at all need be made. Roughly, the black vestigial data should be weighted about three-quarters of its face value as compared with the purple vestigial data. By combining the two sets of approximately weighted data, the mean position of the vestigial locus is found to be 18.5 units to the right of black.

The position of curved is reached by a combination of three sets of weighted data: The most direct data, which needs no correction, is that derived from the vestigial curved cross-over value of 8.2 units based on 1,720 flies. According to this data, the position of curved is at 18.5+8.2, or 26.8 units to the right of black. The next most direct method of location is by reference to purple, the purple curved cross-over value of 19.9 being based on 51,136 flies. This value needs correction according to the probable coincidence of 70 by the addition of 1.5 units, which gives a locus 21.4 units to the right of purple, or 27.6 units to the right of black. The number of flies is to be rated at about 70 per cent of its face value, or at about 38,800 flies. The third method is by means of the black curved cross-over value of 22.7 cor-

rected to 26.2 according to the probable coincidence of 100, and weighted at 50 per cent of the 62,679 flies (31,340). The mean position of curved is 27.0 units to the right of black.

The fifth locus to be considered is that of dachs, which lies to the left of black. The dachs black data give a cross-over value of 17.8 corrected to 18.5 according to the probable coincidence of 50 and weighted at about 80 per cent of the 6,725 flies (5,380). The dachs purple value of 19.7 is corrected to 21.1, according to a probable coincidence of 60 and weighted at about 75 per cent of the 1,489 flies (1,150). The locus is thus at 6.2−21.1, or −14.9. The coincidence in the case of dachs vestigial is known to be about 85, so that the value of 29.6 can be corrected accurately to 34.6, corresponding to a locus of −16.1 and weighted at about 30 per cent of the 5,354 flies (1,605). The mean locus of dachs is thus 17.5 units to the left of black, or at −17.5.

With the mapping of the positions of the four genes, black, purple, vestigial, and curved, and also the position of dachs, which lies to the left of black, the skeleton of what may be called the central body of genes is completed. The next step is to tie onto this central group the outlying loci at either end. Of those to the left, streak is the most important locus, and its position is found by combining three sets of data. The streak dachs cross-over value of 12.7, based on 858 flies, needs no correction, since the probable coincidence is under 10 and the correction negligible in amount. The streak black value of 26.0 should be corrected to about 28.2, corresponding to a coincidence of 60, and with the 462 flies weighted at 65 per cent, *i. e*, 307. The most extensive data is that on streak purple, but the 2,665 flies should be weighted at only about 50 per cent of their number, or at 1,333. The coincidence is probably about 90, so that the value of 33.1 becomes 40.4, corresponding to a locus of −34.2. The mean position of streak is at −31.1.

The position of star is 15.9 units to the left of streak or at −47.0, according to the star streak cross-over value of 15.9, which needs no correction. The star dachs value of 27.3 is corrected to 28.8, according to the probable coincidence of 30. The locus indicated is 46.3 units to the left of black. The 3,472 flies may be rated at about 80 per cent of their number, or at 2,778. The star black value of 37.9 probably represents a total of 46.6 per cent of crossing-over, with a coincidence of 90, and the 16,507 flies may be rated at about 35 per cent of the number, or at 5,775. The mean position of star is thus at −46.5.

The location of all the right end is dependent on speck, which is itself mainly dependent on curved. The curved speck value is 30.5 and the coincidence is known to be very low in this region, probably not over 20, so that the corrected value is about 31.0. Because of this low coincidence a relatively large weighting can be assigned (85 per cent = 8,540). The vestigial speck data furnish 2,054 flies weighted

at about 80 per cent, and a cross-over value of 35.9 corrected to 37.6 according to the probable coincidence of 30. The net coincidence in the case of purple speck is about 50, so that the value of 45.7 may be corrected to 53.6, with a locus of 59.8 The weighting corresponding to this distance and coincidence is about 70 per cent, or there is the equivalent of 8,390 flies. The mean position for the locus of speck is at about 58.6 units to the right of black.

The establishment of the foregoing loci complete what may be called the "triangulation" for the map of the second chromosome. The remaining loci are filled in secondarily with relation to one or more of these bases, or in the case of a few, the relation to still other loci must be considered. Thus the position of morula is dependent primarily on the position of arc, which must first be located. The mean position of arc is found by means of the arc speck value of 5.9 based on 2,625 flies, the purple arc value corrected to 44.2 (C=40) and weighted at 1,838. the black arc value corrected to 52.1 (C=70) and weighted at 3,038, to be 51.9 units to the right of black or −6.7 from speck.

The position of morula at black + 59.8 is based entirely on the arc morula value of 7.9 found from 6,794 flies.

The remaining loci will be treated very briefly in the order of their appearance.

The locus of olive is not exactly known, nor is it important. The probability is that it lies to the right of speck and not more than a unit distant, or at 106.1 referred to star.

The locus of truncate is best found from the star truncate value of 27.1 corrected to 28.0 (C=25), which give a position 28 units to the right of star.

The locus of balloon is 0.4 unit to the right of speck (S′+105.5), based on 462 flies.

The locus of the lethal hypothecated in the chromosome homologous to that carrying truncate (lethal T′) is probably within 15 units of the truncate locus as judged from the proportion of wild-type and truncate flies in the highly selected stocks.

The locus of blistered is approximately 2 units to the left of speck (or at S′ + 103) as deducted from the qlistered speck cross-over value and certain later indications.

The locus of jaunty is very close to that of black, probably 0.2 unit to the right (S′+46.7), on the basis of one questionable cross-over in Muller's progeny tests.

Strap, antlered, and nick are probably allelomorphs of vestigial with the same locus (S′+65.0). If they are not allelomorphs, then their loci are so close to that of vestigial that the interval is negligible.

The locus of gap is suspected of being in the neighborhood of curved.

The locus of comma is perhaps ± 15 units from that of squat (Sq′=S+35.5), as judged roughly from the distribution of squat and comma.

The locus of apterous is about 2 units to the right of black, or at approximately 48.8.

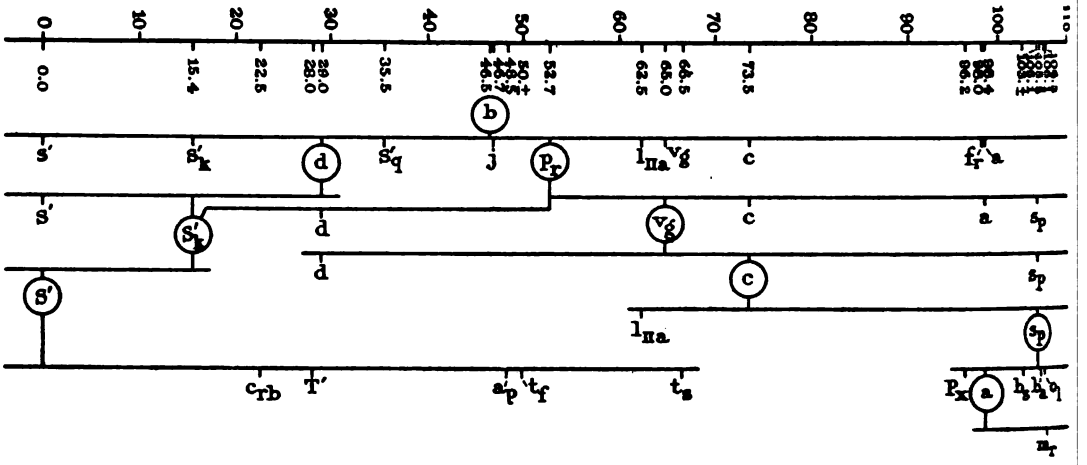
Cream II and patched were found to be linked, but their positions with respect to the other loci were not found.

The position of trefoil is around 50 units from star.

The corrected star cream *b* value of 22.5 gives the locus of cream *b* directly.

The gene for pinkish is located in the far right end of the chromosome, but the locus has not been accurately determined.

The plexus speck value of 8.7 is at present the only acceptable information on the precise location of plexus, which is thus at a locus of 96.2.



TEXT-FIGURE 87.—Constructional map of second chromosome, giving bases of reference and indicating various cross-over values used in calculating mean position for each locus.

The locus of limited is either the same as that of morula, which is possible, or is slightly to the right.

The black fringed value of 42.5 is almost the same as the black arc value of 42.6, so that we may place fringed at 98.0.

The locus of dachs-lethal is probably the same as that of dachs, 29.0 (dachs-deficiency); but if this is not the case, then the locus is so close to that of dachs that the interval is negligible.

Squat gave 11.0 per cent of crossing-over with black and can therefore be mapped at 35.5.

Lethal II*a* gave a value of 13.0 with black, and a value of 8.7 with curved. In the first case there were 166 flies, indicating a position of 59.5; and in the second case 249 flies, indicating a position at 64.8. The mean position is thus 62.7.

The position of telescope is known only from the star telescope back-cross value of 44.4, which indicates a locus at about 66.5 (C = 100).

The loci of the various mutant genes with respect to black as a base of reference have just been found. In some regards it is more convenient to renumber these loci so that the left-most (star) is taken as

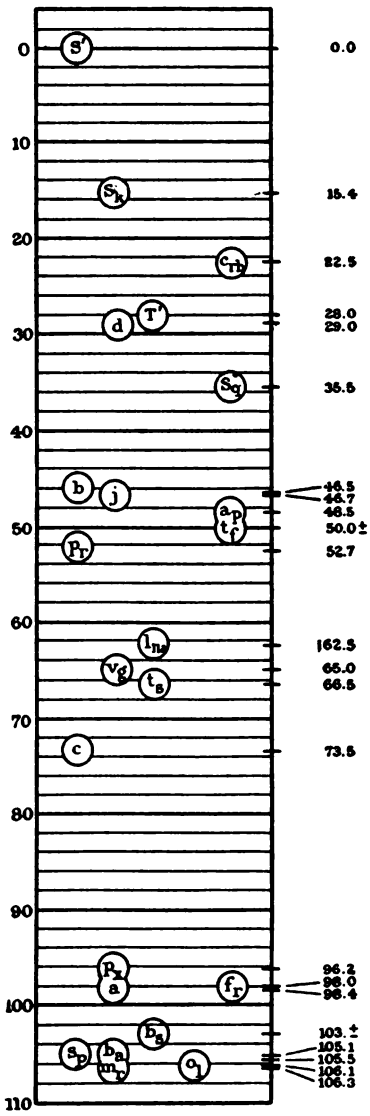
the zero-point and the others have consecutive numbers in a single series. The map made in this way has already been given on page 127.

In using such a map one should keep in mind both the locus as given and the manner in which that locus has been established, since this largely determines not only the accuracy but also the significance of any particular location.

A type of diagram which is capable of representing fully the relationship of each locus to the other loci is given in text-figure 87.

This diagram could be further elaborated by making the heaviness of line correspond to the accuracy of data, and by giving, besides the final, the reference-base positions. Thus *pr vg* gives a locus of vestigial at *b*+18.0, while the corrected black vestigial data indicate a locus of vestigial at *b*+18.9, while the locus actually given in the diagram is the mean position for vestigial at 18.5

The type of map which is in daily use in our laboratory is that given in text-figure 88, in which the loci are further classified according to the value of the character, etc. Thus, the mutants of first rank in value are made conspicuous and insured first consideration by being lined up at the extreme left edge of the space. The mutants nearly as good, but whose usefulness is restricted in one or another respect, are spaced next in order. Still further to the right are those whose loci are not well established or whose characteristics are such that they are useful only in experiments of a very special nature. At the extreme right are the mutants no longer available, because the stocks have been lost or discarded. This type of map can be kept subject to continuous changes in the valuations or the locations of the different mutants by drawing the map-scale on a soft board and mounting the symbols for each mutant on the head of a thumb-tack.



TEXT-FIGURE 88.—Working and valuation map of the second chromosome. The loci mapped at the left margin represent the most valuable mutants, those farther to the right progressively less useful. Those next the right margin are mutants no longer extant.

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III.

INHERITED LINKAGE VARIATIONS IN THE SECOND CHROMOSOME.

By A. H. Sturtevant.

INHERITED LINKAGE VARIATIONS IN THE SECOND CHROMOSOME.

By A. H. Sturtevant.

INTRODUCTION.

The data presented in this paper demonstrate the existence of two genes that influence the amount of crossing-over in the second chromosome of *Drosophila melanogaster* (*ampelophila*).[1] These two genes were both found in the same female, that came from a stock collected in Nova Scotia. Each of the genes, in females heterozygous for it, decreases the amount of crossing-over in the region in which it lies. One of them (the other has not been tested) produces no appreciable effect on crossing-over in females homozygous for it. These results are both paralleled by the effects produced on the third chromosome by a gene in that chromosome. The latter case is discussed briefly. An account is also given of a race in which the amount of crossing-over in one region of the second chromosome is increased. This last case is not yet fully worked out.

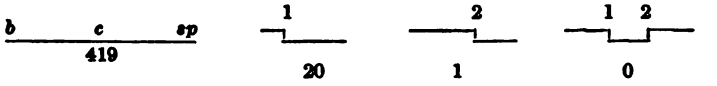
NOVA SCOTIA CHROMOSOME.

The two loci, vestigial and speck, usually show about 37 per cent of crossing-over, as appears from the summaries here presented by Bridges and Morgan. In September 1913, the writer mated a wild female, of a fresh stock collected by Miss E. M. Wallace at Liverpool, Nova Scotia, to a vestigial speck stock that had been used in making the crosses reported by the writer (1915), and had in those crosses given the usual result. A single F_1 female from this mating was mated back to three vestigial speck males of the above stock to produce culture 7 of this paper. The result of this mating was 55 wild-type offspring and 44 vestigial specks—no cross-overs (see Appendix). Two of the wild-type daughters were mated to vestigial speck brothers, to produce cultures 68 and 69. These produced 2 cross-overs among 136 and 2 among 120 offspring, respectively. The same type of mating was repeated in the next generation, in cultures 104, 105, 106, 110, 113, and 114. In 104 and 105 great difficulty was experienced in classifying speck (the only time I have ever noticed such a difficulty with this character), and the two cultures were unfortunately discarded without any attempt being made to see wherein the difficulty

[1] A preliminary note on this case has already been published (Sturtevant, 1917). It has also been discussed by Morgan, Sturtevant, Muller, and Bridges (1915), Muller (1916), and elsewhere.

lay. The other four cultures gave again few or no cross-overs; and this type of mating was carried on for two additional generations with the same result (see Appendix). It is evident that in every case the tested female has at least a part of the "wild-type" second chromosome present in the female of culture 7 and derived from the Nova Scotia stock. That this chromosome is really responsible for the result has been shown in several ways, as follows:

A wild-type female from 69 was mated to 4 black curved speck males of an unrelated stock. The F_1's were wild-type and speck in approximately equal numbers, as would be expected. Except for the rare cross-overs, all the not-speck flies should have carried the Nova Scotia chromosome; and all were heterozygous for black, curved, and speck. Two such wild-type females were back-crossed to black curved speck males (cultures 171 and 172). They gave similar results, which, when added show the following relations:



Total 440.

Here we have the same reduction of curved speck crossing-over that has already been observed for vestigial speck, which includes the curved speck region, and also a reduction of the black curved crossing-over. Experiments exactly analogous to these have been carried out with curved speck, black purple vestigial arc speck, black purple curved, star black purple curved speck, black purple curved morula, star black plexus, and other stocks, always with the same result—greatly reduced crossing-over when the Nova Scotia chromosome is present (see table 1). In several of these cases the chromosome in

TABLE 1.—*Tests of females with one original Nova Scotia chromosome.*

Loci.	0	1	2	3	4	1, 2	Total.
S' b p_7 *c* s_p.....	222	0	0	1	0	0	223
S' b p_2.....	384	0	12	.....	.....	0	396
b p_7 v_g a_7 s_p.....	1,063	0	10	0	1	0	1,094
b p_7 *c*.....	9,422	26	82	0	0	3	9,533
b p_7 *c* m_7.....	2,108	1	42	0	0	1	2,152
b c s_p.....	419	20	1	.....	.....	0	440
b m_7.....	272	5	.....	.....	.....	.....	277
b b_a.....	1,607	104	.....	.....	.....	.....	1,711
v_g s_p.....	1,183	4	.....	.....	.....	.....	1,187
c s_2.....	1,171	1	.....	.....	.....	.....	1,172

question was transmitted through males, instead of females, as above, but this did not in any way affect the result.

Two wild-type females from 110 were mated to black balloon males of an unrelated stock. All the offspring were, as expected, wild-type in appearance. One half of them should have contained the "Nova Scotia" chromosome, the other half should have received the vestigial speck chromosome, and therefore should have given the usual result

for black balloon (48 per cent). 12 of these females were back-crossed to black balloon males, and gave the two types of results shown in table 2.

TABLE 2.

Culture. No.	No. of Offspring.	b ba cross-overs.
		p. ct.
201	131	3.8
203	203	7.9
204	201	9.0
206	242	6.6
207	145	3.4
209	186	2.7
210	256	5.9
211	347	6.9
202	303	41.3
205	386	54.1
208	224	44.6
212	402	49.3

The experiments described above demonstrated that the unusual result is produced when the Nova Scotia chromosome is present; the black balloon result and other similar ones show that offspring of individuals bearing such a chromosome may give the usual result, these evidently being the offspring that do not receive the chromosome in question. Table 1 shows the results obtained from females bearing one Nova Scotia chromosome.

Since there is here a total of only about 1.5 per cent crossing-over between star and speck, it follows that we have almost certainly been dealing throughout with a second chromosome derived entirely (or at least all of it between star and speck) from the original Nova Scotia stock.

In culture 193 a female heterozygous for curved and speck and for the Nova Scotia chromosome was mated to a curved speck male. A speck female, produced as the result of crossing-over between curved and speck (and therefore bearing the original Nova Scotia chromosome, minus its speck end), was mated to a curved male of stock. Two daughters of the latter culture (in 283 and 284) gave 1 cross-over between curved and speck in 505 offspring. The results obtained with this Nova Scotia chromosome, from which the speck end had been removed, are shown in table 3. Evidently the speck end of the chromosome is not responsible for the unusual results.

TABLE 3.—*Tests of females with one Nova Scotia chromosome, the speck end of which has been replaced.*

Loci.	0	1	2	3	4	1, 2	Total.
b p_r v_g a_r s_p.....	277	0	·5	0	0	1	283
b p_r *c*.....	478	1	6	.....	.....	0	485
b s_p.....	565	7	.....	.....	.....	.....	572

As will be shown below, the Nova Scotia chromosome was ultimately separated into two parts, the separation-point being between purple and vestigial. Tests (see table 16) were made of females in which both parts were present, but were each united to parts of "normal" chromosomes. Culture 778 was of this nature; and 786 and 787 contained daughters of 778 in which the original Nova Scotia chromo-

some had been reconstructed by crossing-over. Table 4 presents the results from these two cultures. The combined data from tables 1, 3, and 4 are summarized in table 5. Figure 1, second line, is a map based on this table.

TABLE 4.—*Tests of females with one reconstituted Nova Scotia chromosome.*

Loci.	0	1	2	3	Total.
b p_r *c* s_p.....	549	0	6	0	555

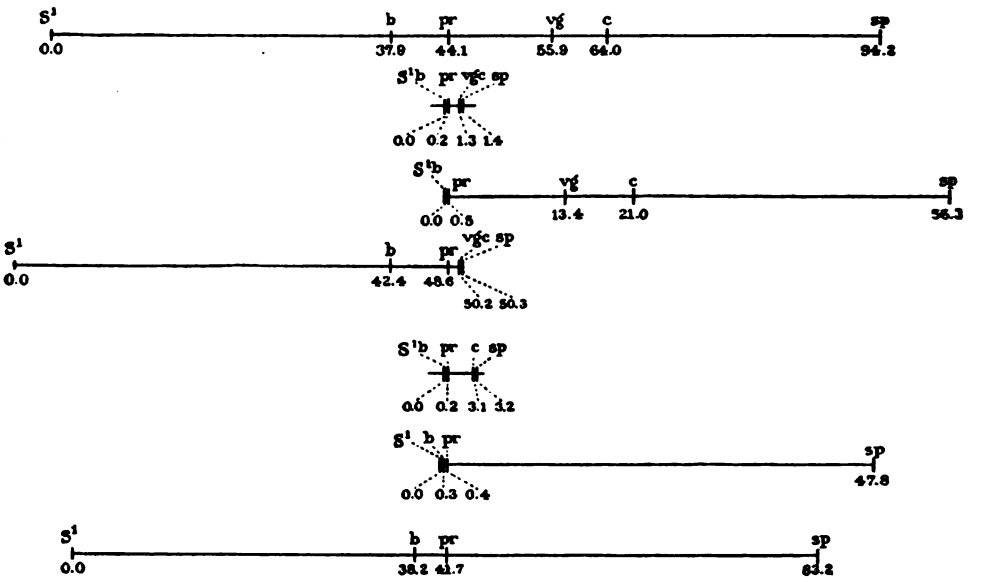


FIG. 1.—Maps based on table 25. The first corresponds to the first column of the table, the second to the second column, etc.

The star black plexus data given here show an unexpectedly high percentage of crossing-over between black and plexus. The data should perhaps not have been included, as there is reason to believe that another gene affecting crossing-over may have been present (see below, p. 324). For this reason plexus has not been entered on the map in figure 1.

The black balloon percentage (6.1) is unexpectedly higher than black speck (1.1). As may be seen from the account here given by Bridges and Morgan, speck and balloon certainly give less than 1 per cent crossing-over in ordinary flies. Unless some complication is here present, balloon must be to the right of speck, and the speck balloon region must give more crossing-over than usual in the presence of a Nova Scotia chromosome.

Table 6 shows the results obtained from second broods,[1] produced by females containing a Nova Scotia chromosome. These data were

TABLE 5.—*Tests of all females bearing one Nova Scotia chromosome (region from star almost to speck).*

Loci.	0	1	Total.	Percentage.
S' b.....	619	0	619	0.0
S' p_r.....	223	0	223	0.0
S' c.....	221	1	223	0.4
S' p_s.....	384	12	396	3.0
S' s_p.....	222	1	223	0.4
b p_r.....	14,293	33	14,326	0.2
b v_g.....	1,362	16	1,378	1.2
b c.....	13,203	185	13,388	1.4
b p_s.....	384	12	396	3.0
b m_r.....	2,381	48	2,429	2.0
b s_p.....	3,117	51	3,168	1.6
b b_s.....	1,607	104	1,711	6.1
p_r v_g.....	1,362	16	1,378	1.2
p_r c.....	12,807	141	12,948	1.1
p_r m_r.....	2,109	43	2,152	2.0
p_r s_p.....	2,133	23	2,156	1.1
v_g s_p.....	2,560	5	2,565	0.2
c m_r.....	2,152	0	2,152	0.0
c s_p.....	2,892	3	2,895	0.1

collected in order to find out if there is a change in the linkage value as a female grows older. The percentages are so small, however, that a comparison with first broods can give no significant result. The small percentages also make impossible a satisfactory study of coincidence in tables 1, 3, and 4.

TABLE 6.—*One original Nova Scotia chromosome, second broods.*

Loci.	0	1	Total.	Percentage.
S' b.....	222	0	222	0.0
S' p_r.....	221	1	222	0.4
S' c.....	219	3	222	1.3
S' s_p.....	219	3	222	1.3
b p_r.....	2,107	9	2,116	0.4
b c.....	2,084	32	2,116	1.5
b m_r.....	936	12	948	1.3
b s_p.....	219	3	222	1.3
p_r c.....	2,087	29	2,116	1.4
p_r m_r.....	937	11	948	1.2
p_r s_p.....	220	2	222	0.9
c m_r.....	948	0	948	0.0
c s_p.....	430	0	430	0.0

Culture 69, referred to above (p. 307), contained a female of the constitution $\frac{\textit{Nova Scotia}}{v_g \qquad s_p}$, mated to a v_g s_p male. One vestigial male, produced by crossing-over and possessing, presumably, only the ex-

[1] "First brood" and "second brood" are terms applied to the offspring produced when a female is kept in one bottle for 9 or 10 days (first brood), and then transferred to another bottle for a second period of 9 or 10 days (second brood). The division is an arbitrary one, and does not correspond to any "rhythm" in the production of eggs.

treme speck end of the Nova Scotia chromosome, was mated to a speck female of stock. The not-speck offspring produced were $\frac{v_g}{s_p}$, with a small piece of the Nova Scotia chromosome opposite s_p. Four females of this constitution were tested, in cultures 166, 167, 168, and 169, by mating to vestigial speck males. The results are shown in table 7.

TABLE 7.

Culture.	0		1 +		Total.
	s_p	v_g	v_g s_p		
166	85	60	46	24	215
167	112	92	42	46	292
168	48	62	46	30	186
169	89	98	41	45	273
Total.....	334	312	175	145	966
	646		320		.....
Percentage...	.....		33.1		.....

These data give approximately the usual value for vestigial speck crossing-over, and therefore agree with the data previously presented in indicating that there is no effect on crossing-over produced by the extreme right-hand end of the Nova Scotia chromosome.

TESTS OF CROSS-OVERS.

When the earlier experiments with the Nova Scotia chromosome were carried out, only that part of the chromosome from black to speck or balloon was studied. Numerous tests of cross-overs were made, in order to find out what part of the Nova Scotia chromosome was responsible for the unusual ratios. The result obtained was that that part of it that lies to the left of a point between purple and vestigial gave approximately the ratios found in ordinary stocks; but that part of it that lies to the right of this point between purple and vestigial and to the left of a point between curved and speck gave what seemed at first to be the same ratios as those given by the whole Nova Scotia chromosome. It was therefore concluded that the peculiarity was due to one gene, located between purple and speck. But it soon appeared that this right-hand end was giving a little more crossing-over in the black-curved region than was the whole Nova Scotia chromosome. By this time star had become available, and it was now found that star and black gave no cross-overs in the presence of the whole chromosome, but gave the usual 40 per cent in the presence of the right-hand end. Tests were then made of the left-hand end again, and star and black were found to give no cross-overs, while

black-purple gave a greatly reduced value. It therefore follows that the original Nova Scotia chromosome contained two factors:

$C_{II\,l}$, located to the left of purple,[1] which makes star black 0.0, and reduces black-purple.

$C_{II\,r}$, located between purple and speck,[1] which greatly reduces the whole purple speck region.

RIGHT-HAND END OF NOVA SCOTIA CHROMOSOME ($C_{II\,r}$).

Culture 171 contained a female with an original Nova Scotia chromosome and a black curved speck chromosome $\frac{C_{II\,l}\ C_{II\,r}}{b\ c\ s_p}$, mated to a black curved speck male. It is included in tables 1 and 5. A black female, produced by crossing-over, must have had the right end of the Nova Scotia chromosome, but not the left $\left(\frac{b\ C_{II\,r}}{b\ c\ s_p}\right)$. This female, in culture 226, was mated to stock curved speck males, and produced 146 offspring without a cross-over between curved and speck. A wild-type daughter, $\frac{b\ C_{II\,r}}{c\ s_p}$, was mated to stock black purple curved males (culture 277). Among 105 offspring, 3 were cross-overs between black and curved. One of the three, a wild-type daughter, $\frac{C_{II\,r}}{b\ p_r\ c}$, was mated, in culture 318, to stock black purple curved males. Wild-type daughters, of the same constitution, were again mated to black purple curved males in cultures 354 and 355. The results were as shown in table 8.

TABLE 8.

Culture.	0	1	2	1, 2	Total.
318	227	13	3	0	243
354	211	11	4	0	226
355	184	11	2	2	199
Total.....	622	35	9	2	668
Percentage....	93.1	5.2	1.3	0.3	.....

A number of other cultures (see table 9) were made in which this same right-hand end of the Nova Scotia chromosome was tested. All were descended from 226, 277, and 318. The results are included in tables 10 and 11. All these cultures agreed in showing that for the purple-to-speck region we have a result not very different from that given by the whole Nova Scotia chromosome; but for the black purple region the result is not very different from the usual one.

[1] Tests made more recently, in connection with studies of $C_{III,\,II}$ (see below), indicate that $C_{II\,l}$ is probably to the left of black, and that $C_{II\,r}$ is certainly to the left of plexus.

Numerous other tests have been made of the right end of the Nova Scotia chromosome. Table 9 gives a list of the different cross-overs tested, together with the cultures derived from those sources. In addition, there are a number of cultures (including all those in which the character star was tested) in which the origin of the $C_{II\,r}$ segment is uncertain, because it has been passed through females homozygous for $C_{II\,r}$ from different sources (see below).

TABLE 9.—*Tests of right-hand end of Nova Scotia chromosome.*

Culture in which cross-over occurred.	Loci between which cross-over occurred.	Cultures representing this piece of the Nova Scotia chromosome (see Appendix).
171	$b\ c$.....	226, 277, 318, 351, 352, 353, 354, 355, 377, 379, 380, 404, 416, 417, 430, 431, 432, 433, 446, 448, 449, 450, 467, 468, 469, 470, 471, 472, 473, 480, 495, 496, 500, 511, 512, 513, 517
524 *N*	$p_r\ v_g$.....	547
524 *N*	$p_r\ v_g$.....	545, 546, 569, 622, 686, 687, 719, 721, 723, 724, 725, 763
685	$p_r\ c$.....	707, 708, 709

These cultures all contain only that part of the Nova Scotia chromosome that lies to the right of a point between black and curved (171 series), purple and vestigial (two 524 N series), or purple and curved (685 series). Since they all agree in the results produced, we may conclude that the gene responsible for these results is located somewhere to the right of purple. In dealing with the original Nova Scotia chromosome we found that removal of the speck end made no difference in the ratios given. It was therefore to be expected that the right-hand piece would show the same relation, *i. e.*, that $C_{II\,r}$ is between purple and speck. The following data show that such is, in fact, the case.

Culture 546, of the second 524 N series, contained a female of the constitution $\frac{b\ p_r\quad C_{II\,r}}{a_r\ s_p}$, mated to black purple arc speck males. By double crossing-over, a female was produced of the constitution $\frac{p_r\ C_{II\,r}\ s_p}{b\ p_r\ a_r\ s_p}$. This female, in which the extreme speck end of the old Nova Scotia chromosome had been lost, was mated to black purple vestigial arc speck males, in culture 570. All later cultures of the second 524 N series received their $C_{II\,r}$ from this female. They gave essentially the same results as the other $C_{II\,r}$ cultures, and have therefore been included in tables 10 and 11. The fourth line of figure 1 represents a map based on table 11.

TABLE 10.—C_{II_r}.

Loci.	0	1	2	3	4	1,2	1,3	2,3	2,4	3,4	1,2,3	Total.
S' b p_r c s_p	805	657	86	17	1	32	11	2	1	1	2	1,615
S' b p_r s_p	420	297	26	10	....	9	3	2	....	....	0	767
b p_r v_g a_r s_p	198	10	2	0	0	0	0	0	0	0	0	210
b p_r v_g s_p	101	16	0	0	....	0	0	0	....	....	0	117
b p_r c m_r	700	39	26	0	....	0	0	0	....	....	0	765
b p_r c s_p	943	59	12	0	....	1	0	0	....	....	0	1,015
b p_r c	7,009	427	103	....	....	10	....	....	....	....	....	7,549
b p_r s_p	888	86	61	....	....	11	....	....	....	....	....	1,046
b p_r	578	25	....	....	....	....	....	....	....	....	....	603
b v_g s_p	141	15	0	....	....	0	....	....	....	....	....	156
b c	790	65	....	....	....	....	....	....	....	....	....	855
b m_r	690	44	....	....	....	....	....	....	....	....	....	734
p_r c	609	6	....	....	....	....	....	....	....	....	....	615
c s_p	146	0	....	....	....	....	....	....	....	....	....	146
Second broods.												
S' b p_r c s_p	435	292	32	16	0	9	5	3	0	0	2	794
S' b p_r s_p	163	139	11	2	....	3	2	0	....	....	0	320
b p_r c	830	70	20	....	....	3	....	....	....	....	....	923
b p_r s_p	234	50	35	....	....	4	....	....	....	....	....	323
b v_g s_p	217	5	0	....	....	0	....	....	....	....	....	222

TABLE 11.—C_{II_r}.

Loci.	0	1	T.	Percentage.	Loci.	0	1	T.	Percentage.
S' b	1,371	1,011	2,382	42.4	*Second broods.*				
S' p_r	1,275	1,107	2,382	46.5	*S' b*	662	452	1,114	40.6
S' c	851	764	1,615	47.3	*S' p_r*	630	484	1,114	43.5
S' s_p	852	763	1,615	47.2	*S' c*	452	342	794	43.0
b p_r	12,843	844	13,687	6.2	*S' s_p*	452	342	794	43.0
b v_g	440	43	483	8.9	*b p_r*	2,390	192	2,582	7.4
b c	10,920.	879	11,799	7.4	*b v_g*	217	5	222	2.3
b m_r	1,390	109	1,499	7.3	*b c*	1,562	155	1,717	9.1
b s_p	4,470	456	4,926	9.2	*b s_p*	1,498	161	1,659	9.7
p_r v_g	325	2	327	0.6	*p_r c*	1,668	49	1,717	2.9
p_r c	11,368	191	11,559	1.6	*p_r s_p*	1,370	67	1,437	4.7
p_r m_r	739	26	765	3.4	*v_g s_p*	222	0	222	0.0
p_r s_p	4,634	136	4,770	2.9	*c s_p*	794	0	794	0.0
v_g s_p	483	0	483	0.0					
c m_r	765	0	765	0.0					
c s_p	2,773	3	2,776	0.1					

The second-brood data are not very conclusive. Star-black is perhaps lower than in first broods; but the black purple curved combinations, for which there is more adequate data, all give a slight increase. As is shown by the data presented by Plough (1917), more exact methods are needed in studying this problem. An experiment with C_{II_r} is now planned in which the females will be transferred every two days. Until data from such an experiment are available further discussion would be out of place.

LEFT-HAND END OF NOVA SCOTIA CHROMOSOME ($C_{II\,l}$).

Culture 678 was derived from a female with an original Nova Scotia chromosome and with black, purple, curved, and morula in its mate, mated to four black purple curved males. The culture is included in the totals given in table 1. It produced three curved flies by crossing-over. These flies must have had the left-hand end of the Nova Scotia chromosome, up to a point between purple and curved; but the right-hand end of the Nova Scotia chromosome had been lost. One of them, a male, was mated to a black purple female that had the right-hand end of the Nova Scotia chromosome ($C_{II\,r}$). A wild-type daughter was mated to black purple curved morula, and gave results that will be discussed below (see table 16). A curved-morula son, that must again have had the left-hand end of the Nova Scotia chromosome, was mated to a similar black purple female; and a wild-type daughter was once more back-crossed to black purple curved morula in culture 752. This time, however, it was decided to get the influence of the left-hand piece of the Nova Scotia chromosome without the presence of the right-hand end. A curved morula male from 752 was accordingly mated to a star black female of an unrelated stock. A daughter, of the constitution $\frac{S'\ \ b}{C_{III}\ \ c\ \ m_r}$, with the end of the Nova Scotia chromosome opposite star and black, was mated to black purple curved speck males, in culture 776. The result was 106 non-cross-overs, 0 cross-overs between star and black, and 38 cross-overs between black and curved. Further tests with descendants of 776, in which the same piece of the Nova Scotia chromosome was present, gave the results shown in table 12 (culture 776 itself is included).

TABLE 12.—$C_{II\,c}$.

Loci.	0	1	2	3	1,2	2,3	T.
*$S'\ b\ c\ s_p$...	1,006	0	455	649	0	141	2,251
$S'\ b\ c$.....	200	0	54	.....	0	....	254
$b\ p_r\ c\ s_p$...	91	0	35	68	0	6	200
$b\ p_r\ s_p$.....	138	1	109	.....	1	....	249
Second broods.							
*$S'\ b\ c\ s_p$...	576	0	216	337	0	41	1,170
$S'\ b\ c$.....	96	0	11	.....	0	....	107
$b\ p_r\ c\ s_p$...	137	0	36	82	0	6	261

* Done with H. H. Plough, and already reported by him (Plough, 1917).

Clearly we have here the same results for star purple as in the case of the original Nova Scotia chromosome; but for purple speck we have nearly the usual result.

Culture 259 contained a female of the constitution $\frac{C_{III}\ \ C_{II\,r}}{b\ \ p_r\ \ v_g\ \ a_r\ \ s_p}$ (original Nova Scotia chromosome), and two stock $b\ p_r\ v_g\ a_r\ s_p$ males.

One vestigial (arc) speck male was produced by crossing-over. He must have had the left-hand end only of the Nova Scotia chromosome, $\frac{C_{II\,l}\ \ v_g\ \ a_r\ \ s_p}{b\ \ p_r\ \ v_g\ \ a_r\ \ s_p}$. He was mated to a black female of stock, and two wild-type daughters, $\frac{C_{II\,l}\ \ v_g\ \ a_r\ \ s_p}{b}$, were tested, in cultures 328 and 329. They gave the results shown in table 13.

TABLE 13.

Culture.	0		1		2		1, 2		Total.
	$v_g\ s_p$	b	+	$b v_g\ s_p$	v_g	$b\ s_p$	s_p	$b\ v_g$	
328	77	75	28	5	79	63	8	6	341
329	44	40	13	7	31	43	2	1	181
	121	115	41	12	110	106	10	7	522
Total.....	236		53		216		17		
Per cent..	45.2		10.2		41.4		3.3		

These data have been added to those of table 12 in the summary for $C_{II\,l}$, table 14. The corresponding map is shown in the third line of figure 1.

TABLE 14.—$C_{II\,r}$, *summary.*

Loci.	0	1	Total.	Percentage.
$S'\ b$.....	2,505	0	2,505	0.0
$S'\ c$.....	1,855	650	2,505	25.9
$S'\ s_p$.....	1,147	1,104	2,251	49.0
$b\ p_r$.....	447	2	449	0.5
$b\ v_g$.....	452	70	522	13.4
$b\ c$.....	2,014	691	2,705	25.4
$b\ s_p$.....	1,497	1,476	2,973	49.7
$p_r\ c$.....	159	41	200	20.5
$p_r\ s_p$.....	236	213	449	47.4
$v_g\ s_p$.....	289	233	522	44.6
$c\ s_p$.....	1,587	864	2,451	35.3
Second broods.				
$S'\ b$.....	1,277	0	1,277	0.0
$S'\ c$.....	1,009	268	1,277	21.0
$S'\ s_p$.....	617	553	1,170	47.3
$b\ p_r$.....	261	0	261	0.0
$b\ c$.....	1,228	310	1,538	20.1
$b\ s_p$.....	760	671	1,431	47.0
$p_r\ c$.....	219	42	261	16.1
$p_r\ s_p$.....	143	118	261	45.2
$c\ s_p$.....	965	466	1,431	32.6

The values obtained from second broods (table 14) run consistently a trifle lower than the corresponding values for first broods. The differences are small in every case; but since all are in the same direction, and since females with neither $C_{II\,l}$ or $C_{II\,r}$ show an age change

in this same direction (Bridges, 1915; Plough, 1917), the decrease is probably significant. More exact methods (see Plough, 1917) are necessary for obtaining clear-cut data on this point, as has already been stated.

Here, as also in the case of $C_{II\,r}$, wherever reliable information regarding coincidence is available, the value is not far from the one found in females that contain neither $C_{II\,l}$ nor $C_{II\,r}$ (see Bridges

TABLE 15.— $\frac{C_{II\,l}}{C_{II\,r}}$

Loci.	0	1	2	3	4	1, 2	Total.
S' b p_r c s_p	659	0	1	24	1	0	685
b p_r c	226	2	10	..	..	0	238
b p_r c m_r	631	0	12	0	..	..	643
Second cultures.							
S' b p_r c s_p.	497	0	0	11	1	0	509

TABLE 16.— $\frac{C_{II\,l}}{C_{II\,r}}$

Loci.	0	1	T.	Percentage.
S' b	685	0	685	0.0
S' p_r	684	1	685	0.1
S' c	660	25	685	3.6
S' p_r	659	26	685	3.7
b p_r	1,563	3	1,566	0.2
b c	1,517	49	1,566	3.1
b m_r	631	12	643	1.9
b s_p	659	26	685	3.7
p_r c	1,520	46	1,566	2.9
p_r m_r	631	12	643	1.9
p_r s_p	660	25	685	3.6
c m_r	643	0	643	0.0
c s_p	684	1	685	0.1
Second broods.				
S' b	509	0	509	0.0
S' p_r	509	0	509	0.0
S' c	498	11	509	2.2
S' s_p	497	12	509	2.4
b p_r	509	0	509	0.0
b c	498	11	509	2.2
b s_p	497	12	509	2.4
p_r c	498	11	509	2.2
p_r s_p	497	12	509	2.4
c s_p	508	1	509	0.2

and Morgan, 1919). But in no case does this include a region in the "sphere of influence" of the cross-over gene present; for in all such regions the percentage of crossing-over is too small to give statistically reliable results.

$$\frac{C_{II\,l}}{C_{II\,r}}$$

Cultures 677 and 678 both contained females, $\frac{b \quad p_r \quad c}{C_{II\,l} \quad C_{II\,r}}$ (678 was also heterozygous for m_r), mated to *b p_r c* males. A cross-over female,

$\frac{b\ p_r\ C_{II\,r}}{b\ p_r\ c}$, from 677 was mated to a cross-over male, $\frac{C_{II\,l}\ c\ m_r}{b\ p_r\ c}$ from 678. The resulting wild-type offspring were $\frac{C_{II\,l}\ c\ m_r}{b\ p_r\ C_{II\,r}}$. A female of this constitution, in culture 713, gave 4.3 per cent crossing-over between *pr* and *c*, none between *b* and *pr* or between *c* and *mr*. The same general method was followed in making up seven other $\frac{C_{II\,l}}{C_{II\,r}}$ females. The same $C_{II\,l}$ *c* chromosome was present in all these females, but C_{II} *r* from different sources was used. The results from these females are given in tables 15 and 16 and the fifth line of figure 1.

These data agree with those obtained from $C_{II\,l}\ C_{II\,r}$, except that $p_r\,c$ shows a slight rise (from 1.1 to 2.9). Owing to the statistical difficulty of handling such small ratios it is not possible to say whether this difference is significant or not until more data can be collected. The point is of interest in its bearing on the mechanism of the action of $C_{II\,l}$ and $C_{II\,r}$, but must be left unanswered for the present.

The second brood data here presented for $\frac{C_{II\,l}}{C_{II\,r}}$ are entirely inadequate for the purpose of detailed comparison with first broods. They do, however, show an increase for $p_r\ c$ over the $C_{II\,l}\ C_{II\,r}$ second broods (from 1.2 to 2.2).

HOMOZYGOUS $C_{II\,r}$.

We have seen that heterozygous $C_{II\,r}$ greatly decreases crossing-over in the region from purple to speck, but does not appreciably affect the region from star to purple. The data now to be presented show that homozygous $C_{II\,r}$ gives a value for purple speck that is very close to that found in "normal" flies, but again does not influence the region from star to purple.

It has so far not been found possible to obtain a chromosome containing $C_{II\,r}$ with vestigial or curved, since heterozygous $C_{II\,r}$ practically prevents all crossing-over in the region in which these three genes are located. For this reason none of the data on homozygous $C_{II\,r}$ deal with loci between purple and speck.

HOMOZYGOUS $C_{II\,r}$ WITH $C_{II\,l}$.

In the course of the experiments with $C_{II\,r}$ a chromosome of the constitution $S'\ b\ p_r\ C_{II\,r}\ s_p$ was obtained.[1] When males with this

[1] This chromosome was derived from culture 570 (see p. 314), in which was a female $\frac{p_r\ C_{II\,r}\ s_p}{b\ p_r\ a_r\ s_p}$. A cross-over in this female gave a $b\ p_r\ C_{II\,r}\ s_p$ chromosome. This chromosome, or a derivative of it, since it had perhaps been passed through a $\frac{C_{II\,r}}{b\ p_r\ C_{II\,r}\ s_p}$ female, was placed opposite star $\left(\frac{S'}{b\ p_r\ C_{II\,r}\ s_p}\right)$ in the females of cultures 696 and 699a. The chromosome referred to above ($S'\ b\ p_r\ C_{II\,r}\ s_p$) was produced by crossing-over in these females and was kept intact thereafter by breeding from *males* heterozygous for it, in which no crossing-over occurred.

chromosome were mated to females of the constitution $\frac{C_{II\,l}\ C_{II\,r}}{b\ p_r\ c}$, the star not-black offspring must have been of the constitution $\frac{S\ b\ p_r\ C_{II\,r}\ s_p}{C_{II\,l}\quad C_{II\,r}}$. Nine such females were tested by mating to $b\ p_r\ s_p$ males and gave the results shown in the first line of table 17. The data in the second row were obtained in the same way, except that no star had been put in the $b\ p_r\ C_{II\,r}\ s_p$ chromosome. The third line represents the offspring of a female (culture 340) of the constitution $\frac{C_{II\,l}\ C_{II\,r}\ s_p}{b\quad C_{II\,r}}$, produced by mating a male $\frac{b\ \ pr\ \ c}{b\quad C_{II\,r}}$ to a female $\frac{C_{II\,l}\ C_{II\,r}\ s_p}{c\quad s_p}$. The males in 340 were black purple vestigial arc speck; since no purples were produced the female must have received a $b\ C_{II\,r}$ chromosome from her father; and since she gave 45.2 per cent crossing-over between black and speck, instead of the 9.0 characteristic of $C_{II\,r}$ females, she must have received from her mother $C_{II\,l}\ C_{II\,r}\ s_p$.

TABLE 17.— $\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$

Loci.	0	1	2	3	1,2	1,3	2,3	1,2,3	Total.
$S'\ b\ p_r\ s_p$...	1,047	3	0	942	0	1	0	2	1,995
$b\ p_r\ s_p$.....	154	0	138	.....	1	....	....	.....	293
$b\ s_p$.....	144	119	.....	.....	....	....	....	.....	263

TABLE 18.— $\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$

Loci.	0	1	Total.	Percentage.
$S'\ b$....	1,989	6[1]	1,995	[1] 0.3
$S'\ p_r$...	1,989	6[1]	1,995	[1] 0.3
$S'\ s_p$...	1,048	947	1,995	47.5
$b\ p_r$...	2,285	3	2,288	0.1
$b\ s_p$....	1,351	1,200	2,551	47.0
$p_r\ s_p$...	1,204	1,084	2,288	47.4

[1] These cross-overs are very doubtful. None of them were tested; and there is a small percentage of error in classifying star flies. Similar apparent cross-overs were obtained in working with $C_{II\,l}$, but all were shown, when tested to see if star was really present or not, to be wrongly classified.

Table 18 and the sixth map of figure 1 summarize the data from these three series of experiments. No second-brood data are available; and the star to purple region gives so few cross-overs that coincidence can not profitably be studied. It is, however, very remarkable that all three cross-overs between black and purple were also cross-overs between purple and speck. More data is needed before we can be sure this is a significant result, since purple and speck themselves cross over so frequently (47.4 per cent).

Comparison of table 18 with table 14 will show that the results given by $\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$ and by $C_{II\,l}$ are almost if not quite the same. That is, homozygous $C_{II\,r}$ gives the same result as no $C_{II\,r}$.

HOMOZYGOUS $C_{II\,r}$—WITHOUT $C_{II\,l}$.

A female $\frac{b\ p_r\ C_{II\,r}}{b\ p_r\ c\ m_r}$, produced by crossing-over in 691*a* $\left(\frac{C_{II\,l}\ C_{II\,r}}{b\ p_r\ c\ m_r}\right)$ was mated to a male $\frac{C_{II\,r}\ s_p}{b\ p_r\ C_{II\,r}\ s_p}$ (a cross-over from 699, *q. v.*, see above. Six wild-type daughters were tested by mating to $b\ p_r\ c\ s_p$ males. Four gave the expected result for $\frac{b\ p_r\ c\ m_r}{C_{II\,r}\ s_p}$ females; and two (745 and 748) gave no curved offspring, so that they must have been $\frac{C_{II\,r}\ s_p}{b\ p_r\ C_{II\,r}}$.

Females 885 to 888 contained a $S'\ b\ C_{II\,r}$ chromosome derived from the $\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$ experiments and a $p_r\ C_{II\,r}\ s_p$ chromosome derived from a stock culture that came from culture 570 (*q. v.*, p. 314). It is quite possible that some or all of these females carried another gene affecting crossing-over ($C_{III,\,II}$—see below); but the results have

TABLE 19.— $\frac{C_{II\,r}}{C_{II\,r}}$

Loci.	0	1	2	3	1,2	1,3	2,3	1,2,3	Total.
$b\ p_r\ s_p$.....	295	9	161	.....	6	.....	.....	.....	471
$b\ s_p$.....	77	74	.....	.....	.....	.....	.....	.....	151
$S'\ b\ p_r\ s_p$[1]..	303	192	16	242	2	156	12	4	917

TABLE 20.

Loci.	0	1	Total.	Percentage.
$S'\ b$.....	573	354	927	38.2
$S'\ p_r$.....	551	376	927	40.6
$S'\ s_p$.....	473	454	927	49.0
$b\ p_r$.....	1,349	49	1,398	3.5
$b\ s_p$.....	851	698	1,549	45.0
$p_r\ s_p$.....	817	581	1,398	41.5

been included because they are the only ones available for S' in the presence of homozygous $C_{II\,r}$. Other work done with $C_{III,\,II}$ makes it probable that this gene would not seriously affect any region except that from purple to curved; and the purple speck values for this experiment agree with those from 745 and 748. Therefore the two results are probably comparable. Both are included in tables 19 and 20 and the last line of figure 1.

[1]May contain $C_{III,\,II}$.

For this combination also no second-brood data is available. Coincidence seems to be of approximately the value that is usual, but can be satisfactorily studied only in the series that may have $C_{III,\,II}$.

The $\frac{C_{II\,r}}{C_{II\,r}}$ ratios are clearly not very different from those obtained with the "usual" second chromosome.

NO TESTS OF HOMOZYGOUS $C_{II\,l}$.

No tests were made of females homozygous for $C_{II\,l}$, because it was hoped that a cross-over would occur that would give a S' C_{III} chromosome, and thus make possible a test of the region in which $C_{II\,l}$ is located. A few attempts were, it is true, made to get a pure stock of $C_{II\,l}$; but no careful records were kept, and these attempts were all unsuccessful. Recent tests show that there is now a lethal gene in the $C_{II\,l}$ chromosome that is being studied, so that it will probably be impossible to obtain homozygous $C_{II\,l}$. It is not certain whether this lethal represents a recent mutation or not.

TESTS SHOWING NO CROSSING-OVER IN MALES.

Very few counts have been made from heterozygous males; but no crossing-over in males has been assumed throughout the work, and has been depended on frequently in keeping stocks and in producing many of the more unusual combinations of $C_{II\,l}$ and $C_{II\,r}$. These matings have never produced flies that seemed to result from crossing-over in males, and have always given in later generations results that are consistent with the view that such crossing-over does not occur. Taking this evidence in connection with the counts given below (table 21), and with the evidence that shows crossing-over not to occur in males of *Drosophila* in any of the chromosomes under any known circumstances,[1] we may safely conclude tnat $C_{II\,l}$ and $C_{II\,r}$ do not cause exceptions to the general rule.

CONSTITUTION OF THE NOVA SCOTIA STOCK.

The original Nova Scotia female had in her second chromosome two factors for decreased crossing-over. It would be of some interest to find out whether or not this condition was widespread in the stock from which she came. Unfortunately the original stock was lost before it was discovered that two factors, instead of one, are responsible for the result. The following tests are therefore not entirely satisfactory.

Three females, from the Nova Scotia stock, were mated to curved speck, and 4, 4, and 1 daughters, respectively, were back-crossed to curved speck. Only a few offspring were counted from each, but enough to show that all 9 females were giving at least 20 per cent of

[1] Except the curious case of "somatic crossing-over" recorded by Muller (1916).

crossing-over. It follows that $C_{II\,r}$ was not present. Three females from Nova Scotia stock were crossed to black vestigial, and daughters

TABLE 21.—*Tests for crossing-over in males.*

Culture.	Crossover constitution.	Mutant genes.	Non-cross-overs.		Cross-overs.	Total.
108	$\frac{C_{II\,l}\ C_{II\,r}}{}$	$\frac{}{v_g\ s_p}$	37	49	0	86
109	$\frac{C_{II\,l}\ C_{II\,r}}{}$	$\frac{}{v_g\ s_p}$	61	52	0	113
			98	101	0	199
621	$\frac{C_{II\,r}}{C_{II\,r}}$	$\frac{b}{p_r\ s_p}$	2	7	0	9
624	$\frac{C_{II\,r}}{C_{II\,r}}$	$\frac{b}{s_p}$	6	5	0	11
			8	12	0	20.
338*a*	$\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$	$\frac{b}{s_p}$	5	8	0	13
338*b*	$\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$	$\frac{b}{s_p}$	58	58	0	116
423	$\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$	$\frac{}{b\ s_p}$	143	146	0	289
424	$\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$	$\frac{}{b\ s_p}$	81	77	0	158
			287	289	0	576
741*a*	$\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$	$\frac{S'\ s_p}{}$	108	112	0	220
741*b*	$\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$	$\frac{S'\ s_p}{}$	85	87	0	172
			193	919	0	392
718	$\frac{C_{II\,r},\ or}{\frac{C_{II\,r}}{C_{II\,r}}}$	$\frac{S'}{s_p}$	88	102	0	190

TABLE 22.

Mother.	Culture.	*b vg* percentage.	No. of offspring.
N.....	230	8.5	258
N.....	231	9.6	270
N.....	232	10.7	140
V.....	233	13.9	202
V.....	234	11.9	168
V.....	235	16.2	191
V.....	236	12.9	295
V.....	237	13.7	269
V.....	238	20.0	135
B.....	239	12.3	235
B.....	240	23.0	161

were back-crossed to black vestigial (cultures 230 to 240 inclusive). The results are shown in table 22.

None of these females had both $C_{II\,l}$ and $C_{II\,r}$, but it is possible that one of the factors may have been present, especially in the offspring of female *N*. These tests show only that the Nova Scotia stock was not homozygous for $C_{II\,r}$, and probably not for $C_{II\,l}$. No other stocks from northern localities have been tested, so that it is impossible to even guess whether or not these factors occur frequently in Nova Scotia or neighboring regions.

ANOTHER SECOND-CHROMOSOME LINKAGE VARIATION.

Cultures 733 and 734, referred to elsewhere, contained females of the constitution $\frac{C_{II\,l}\ C_{II\,r}}{S'\ b\ p_s}$. As was pointed out above, they gave an unexpectedly high percentage of crossing-over for black and plexus. Culture 812, descended from the same culture that produced females 733 and 734, contained a female $\frac{S'\ b\ p_r\ c\ s_p}{C_{III}\qquad C_{II\,r}}$. This female produced 72 offspring, of which none were cross-overs between S' and p_r, or between c and s_p, but 11 were cross-overs between p_r and c. Later descendants of 812, of the constitution $\frac{C_{II\,l}\ C_{II\,r}}{b\ p_r\ c}$, gave this same increased value for $p_r\ c$ without any increase for $b\ p_r$. But it was found impossible to fix this increased value, which fluctuated between the expected value (less than 1 per cent) and 20 to 30 per cent. Several selection experiments have been carried out in an effort to get a stock that would constantly give the high value, but without success. The most recent of these experiments has now been carried through 23 generations of brother-sister matings, always breeding only from those pairs that gave the "high" value for $p_r\ c$. Yet, in the fifteenth generation, occurred a culture that gave only 1 cross-over among 130 offspring, and in the twenty-third was a culture that gave $\frac{6}{176}=3.4$ per cent. The latter value, while slightly higher than is usual for $C_{II}\ C_{II\,r}$, is much lower than the 20 to 30 per cent now given by most of the "high" selected cultures.[1] The nature of this case has not yet been worked out in detail, though culture 812 was counted in December 1915, and the problem has been worked at continuously since that time.

The following points now seem fairly certain, though they must still be checked and extended.

(1) The "high" value is due, in large part, at least, to a dominant gene.

[1] The C_{III} has apparently been lost, by crossing-over, in part of this experiment. But since the values given above are too high for heterozygous $C_{II\,r}$, the discussion given is not affected.

(2) This gene is not in the second chromosome at all, but in the third.

(3) The third chromosome gene is linked to a gene that is lethal when homozygous. This is the reason the very high values could not be fixed.

(4) This gene, called $C_{III,\ II}$, also causes an increase in p, c crossing-over in $C_{II\ r}$ females. Its effect on females of different constitutions with respect to $C_{II\ l}$ and $C_{II\ r}$ is not yet clear.

(5) $C_{III,\ II}$, when heterozygous, *reduces* the amount of crossing-over in the *third* chromosome. Its effect in this respect is similar to, but not identical with, that of C_{III} (see next section, and Muller, 1916). Unlike C_{III}, it "allows" a few cross-overs between sooty and rough; but it causes a reduction of crossing-over farther to the left than does C_{III}.

(6) Females with $C_{III,\ II}$ in one chromosome, and C_{III} in its mate, give nearly the same amount of crossing-over in the third chromosome as do females heterozygous only for C_{III}, or perhaps less in the left-hand regions.

A detailed comparison of the effects of these two genes, a study of their interaction, and also an investigation of the locus of $C_{III,\ II}$ are now under way.

COMPARISON WITH RESULTS OBTAINED FROM C_{III}.

I have shown (Sturtevant, 1913*a*, 1915) that great linkage variations occur in the third chromosome. My own unpublished data and those presented by Muller (1916) show that the case is very similar to that of $C_{II\ r}$. The factor C_{III}, present in the beaded stock and in several

TABLE 23.

Father of tested ♀.	Culture.	No. of offspring.	Percentage of crossing over.	
			$s_s\ e^s$	$e^s\ r_s$
2,568*a*	2,608	291	0.0	0.0
2,568*a*	2,610	284	10.9	14.1
2,568*a*	2,613	197	0.0	[1]0.5
2,568*a*	2,614	252	0.0	0.0
2,568*a*	2,615	83	7.2	20.5
2,568*a*	2,617	216	0.0	0.0
2,568*b*	2,618	201	0.5	0.0
2,568*b*	2,619	193	0.0	0.0
2,568*b*	2,620	156	11.6	17.3
2,568*b*	2,621	110	13.6	20.9
2,568*b*	2,622	187	0.0	0.0
2,568*b*	2,623	143	0.0	0.0
2,568*b*	2,624	224	0.0	0.0

Total—4 high, 9 low.

[1] Probably an error in classification. Such cross-overs are exceedingly rare. This individual was not tested.

stocks derived from it (ebony, spread, eosin), greatly decreases crossing-over in the right-hand end of the third chromosome when it is present in heterozygous form; but this result disappears in flies homozygous for C_{II_r}. Moreover, the gene is itself located in the region in which it produces its greatest effect. The following sample experiment will illustrate its action.

Certain experiments carried out by Dr. C. B. Bridges, in investigating cream III, led to the hypothesis that the eosin stock was impure for C_{III}. Accordingly two males from this eosin stock were mated individually to sepia spineless sooty rough females, and daughters were back-crossed to sepia spineless sooty rough males, with the results shown in table 23. The values for sepia spineless are not given, because sepia was not easily classifiable in the eosin males produced.

There are clearly two quite distinct types of results here. In 9 of the cultures there is less than 1 per cent crossing-over between spineless and rough; in the other 4 there is about 25 to 30 per cent crossing-over between these loci.[1] The results are due to the presence of C_{III} in those females that gave the low result, and its absence in those that gave the high one. That the difference was due to the nature of the third chromosomes derived from the fathers was shown by testing the crossing-over in wild-type daughters of these females. In every case such daughters gave approximately the same results as their respective mothers. Daughters of all but 2615 and 2621 were so tested.

In females homozygous for C_{III} the crossing-over between s_s and e rises to about 40 per cent ($\frac{185}{446} = 41.6$ per cent, in one experiment selected at random), as against about 12 per cent in the absence of C_{III}, and less than 1 per cent when it is present in heterozygous form. This result is in agreement with Muller's (1916) conclusion that homozygous C_{III} results in the production of more crossing-over than occurs in "normal" females.

The C_{III} experiments are still in progress, and will be reported in detail in connection with the other third-chromosome data accumulated in this laboratory. From the above account, however, it may be seen that the parallel between C_{II_r} and C_{III} is very close. The effect of each upon the region in which it lies is shown in table 24. The s_s e values are only approximately correct.

TABLE 24.

	II–p_r s_p	III–s_s e
Usual result.....	46.5	12.0
Heterozygous C.	2.9	0.5
Homozygous C..	41.5	40.0

[1] It will be observed that both males from eosin stock were heterozygous for C_{III}. There was later found to be a lethal near the C_{III}. This, in connection with other results obtained with the eosin stock, suggests that it was a "balanced lethal" stock for the third chromosome (see Muller, 1917). This stock has now died out, so that it is no longer possible to test such a hypothesis.

OTHER CASES OF LINKAGE VARIATIONS.

The cases reported in this paper are not the only ones in which linkage variations are known. As has been pointed out above, there is a gene in the third chromosome that affects the percentage of crossing-over in that chromosome. It has been shown (Morgan, 1912; Sturtevant, 1913*a*; Morgan, Sturtevant, Muller, and Bridges, 1915; etc.) that there is no crossing-over in the male of *Drosophila*, even between loci that give almost 50 per cent of crossing-over in females. The reverse relation—crossing-over in males but not in females—has been shown by Tanaka (1914) to hold for at least two loci in the silkworm moth. Bridges (1915) has shown that the percentage may change with age, and Plough (1917) has shown that it may be changed by temperature. Genetic factors (other than sex) influencing the process are suggested by the results of Baur (1912) with *Antirrhinum*, of Punnett (1913, 1917) with sweet peas, of Tanaka (1913, 1914) with silkworm moths, and of Chambers (1914) with *Drosophila*. In none of these cases is the evidence yet clear enough to warrant detailed discussion.

BEARING OF METHOD ON CHROMOSOME VIEW.

The work reported in this paper deals with the effects on crossing-over produced by certain definite genes. These genes do not, so far as I have been able to discover, produce any visible somatic effects; and their presence can not be detected, except in females, and in females that are heterozygous for other genes in definite regions of the chromosomes, *i. e.*, that are capable of being tested for linkage in those regions. In the case of other females, or of any males, such tests can not be made directly, but only by producing female descendants heterozygous for the necessary genes. The fact that it has been possible to work out in great detail the inheritance of these "invisible" genes and the effects produced by them is a striking illustration of the possibilities of the chromosome view of inheritance and of the advantages of using a rapidly breeding form like *Drosophila*.

The chromosome view itself is perhaps not necessary for the handling of such a case; but the conception of genes that form independent groups that behave as units, the members of which are only separable according to definite rules, *is* necessary. And such a conception, I think, presupposes some material basis for the independent groups. The great body of evidence that points to the chromosomes as forming such a material basis is too familiar to need discussion here.

SIGNIFICANCE OF MAP DISTANCE.

It has often been pointed out (*e. g.*, Sturtevant, 1913, p. 49; Morgan, Sturtevant, Muller, and Bridges, 1915, pp. 67–68) that 1 per cent of crossing-over must not be supposed to represent the same actual morphological distance in different chromosomes or in different regions of the same chromosome. Actual distance is evidently an important factor in the result. Other things being equal, chromosome sections of equal length will give equal percentages of crossing-over; but in no case can we be certain that "other things" *are* equal. The terms "distance" and "percentage of crossing-over" have unfortunately been sometimes used almost as though synonymous, and confusion has perhaps resulted. But it has been recognized from the beginning that different regions might show different frequencies of crossing-over for the same actual length of chromosome.

The results presented in this paper show conclusively that this is the case, as has already been stated (Morgan, Sturtevant, Muller, and Bridges, 1915; Muller, 1916; Sturtevant, 1917). They show that even in the same chromosome pair the percentage of crossing-over shown by different regions is not only not always the same, but is not necessarily even proportional. For example, while S' b remains approximately 40.0, b c may be either 23.0 (neither $C_{II\,l}$ nor $C_{II\,r}$ present), or 7.5 (heterozygous $C_{II\,r}$).

LINEAR ARRANGEMENT OF GENES.

The strongest evidence for the linear arrangement of genes is that derived from crosses in which more than two loci in the same chromosome can be followed. The method of seriating the loci on the basis of such information has been described in detail elsewhere (Morgan, Sturtevant, Muller, and Bridges, 1915; Sturtevant, 1915; Morgan and Bridges, 1916), so need not be discussed here. When the linkage values are changed the question arises: Is the sequence of genes affected? It has already been shown (Bridges, 1915; Plough, 1917) that this sequence is not altered when the amount of crossing-over is changed by age or by temperature. In the case of the genetic changes reported here, the evidence presented in tables, 1, 3, 10, 12, 14, 17, and 19 shows that the sequence found in "normal" females is maintained. There are just three cases in which the data, uncorrected by other data, might lead us to assign a different sequence. These three cases may now be taken up in turn.

(1) In the case of $\frac{C_{II\,l}\ C_{II\,r}}{b\ p_r\ v_g\ a_r\ s_p}$ only one cross-over between b and p_r was obtained; and that was also a cross-over between p_r and v_g. This would lead us to suppose the sequence to be p_r b v_g, were no

other data known. But the other data for $\underline{C_{II\,l}\ C_{II\,r}}$ show conclusively that b and p_r give very little crossing-over (0.2 per cent), while either with v_g, c, or s_p, gives about 1.1 per cent; and v_g and c give only 0.1 or 0.2 per cent with s_p. That is, v_g and c are on the same side of b and p_r. And the extensive data for $b\ p_r\ c$ show that the sequence is $b\ p_r\ c$. Therefore the one individual that suggested the sequence $p_r\ b\ v_g$ must have been a double cross-over.

(2) In the case of $\underline{C_{II\,r}}$ only three cross-overs between c and s_p were obtained. Of these, two were also cross-overs between b and c, while one was not. These data alone would indicate the sequence as $b\ s_p\ c$, instead of the usual $b\ c\ s_p$. No great significance can be attached to the difference between 2 flies and 1 fly among a total of 1,615. In any case, the data suggest a very high coincidence. More data of the same sort will be necessary before this exceptional case can appear significant.[1]

(3) In the case of $\frac{C_{II\,l}\ C_{II\,r}}{C_{II\,r}}$, only 3 cross-overs were observed between b and p_r. All of these were also cross-overs between p_r and s_p.[2] If the coincidence in this case is 100, approximately the value usual for $b\ p_r\ c$, then nearly half of the $b\ p_r$ cross-overs should be also $p_r\ s_p$ cross-overs. Therefore the fact that all 3 were such doubles need not cause surprise; even though, taken alone, it would indicate the sequence as $p_r\ b\ s_p$.

The three exceptional cases are, then, of no great significance, except as indicating rather high coincidence. There are a large number of cases in which the evidence is much clearer and in which the sequence is certainly the same as that usually found.

HOW DO $C_{II\,l}$ AND $C_{II\,r}$ PRODUCE THEIR EFFECTS?

The question of the mechanism whereby the cross-over genes produce their effects is not yet satisfactorily answered. Cytological examination might conceivably furnish the solution, but has not yet been seriously attempted. A study of coincidence might give a clue, but is difficult to make, because of the very small percentages that are concerned.

In the case of $C_{II\,r}$ and C_{III} it is to be noted that two *like* chromosomes cross over freely, while two *unlike* ones do not.[3] While this is only a restatement of the facts, it at least offers an attractive opening for speculation as to the nature of the case.

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[1] In a culture derived from $C_{III,\,II}$ experiments discussed above, a female that was apparently of the constitution $\frac{C_{II\,r}}{b\ p_r\ c\ s_p}$ (without $C_{III,\,II}$) has recently been tested. One s_p daughter was produced. If this record represents what it appears to, the count becomes 2 double cross-overs against 2 single cross-overs.

[2] Two of them were also recorded as cross-overs between S' and b; but this is probably incorrect, as was pointed out above (p. —).

[3] So far as the evidence goes, this is also true for C_{III}, but $\frac{C_{III}}{C_{III}}$ is unknown.

TABLE 25.

Loci.	Normal.	$C_{II\,l}\,C_{II\,r}$	$C_{II\,l}$	$C_{II\,r}$	$\frac{C_{II\,l}}{C_{II\,r}}$	$\frac{C_{II\,l}\,C_{II\,r}}{C_{II\,r}}$	$\frac{C_{II\,r}}{C_{II\,r}}$
$S'\ b$.....	**37.9**	**0.0**	**0.0**	**42.4**	**0.0**	**0.3**	**38.2**
$S'\ p_r$.....	43.7	0.0	.....	46.5	0.1	0.3	40.6
$S'\ c$.....	45.9	0.4	25.9	47.3	3.6	.....	.....
$S'\ s_p$.....	48.3	0.4	49.0	47.2	3.7	47.5	49.0
$b\ p_r$.....	**6.2**	**0.2**	**0.5**	**6.2**	**0.2**	**0.1**	**3.5**
$b\ v_g$.....	17.8	1.2	13.4	8.9	.....	.....	.....
$b\ c$.....	22.7	1.4	25.4	7.4	3.1	.....	.....
$b\ m_r$.....	46.6	2.0	.....	7.3	1.9	.....	.....
$b\ s_p$.....	47.6	1.6	49.7	9.2	3.7	47.0	45.0
$b\ b_a$.....	48.1	6.1	.....	.....	.....	.....	.....
$p_r\ v_g$.....	11.8	1.2	.....	0.6	.....	.....	.....
$p_r\ c$.....	**19.9**	**1.1**	**20.5**	**1.6**	**2.9**	.....	.....
$p_r\ m_r$.....	.....	2.0	.....	3.4	1.9	.....	.....
$p_r\ s_p$.....	46.5	1.1	47.4	2.9	3.6	**47.4**	**41.5**
$v_g\ s_p$.....	35.9	0.2	44.6	0.0	.....	.....	.....
$c\ m_r$.....	.....	0.0	.....	0.0	0.0	.....	.....
$c\ s_p$.....	**30.2**	**0.1**	**35.3**	**0.1**	**0.1**	.....	.....
Total[1]..	94.2	1.4	56.3	50.3	3.2	47.8	83.2

[1] $S'\ b + b\ p_r + p_r\ c + c\ s_p$, except in the last two columns, where $p_r\ s_p$ is used. Probably all but the second and fifth columns are too low, since no correction has been made for unobservable double cross-overs.

SUMMARY.

Two genes that affect the amount of crossing-over in the second chromosome are discussed. Females of various constitutions with respect to these genes give the results shown in table 25 and figure 1. $C_{II\,l}$, located somewhere to the left of purple, decreases the amount of crossing-over between star and purple in females heterozygous for it. $C_{II\,r}$, located between purple and speck, reduces the amount of crossing-over between purple and speck in females heterozygous for it; but females homozygous for $C_{II\,r}$ show the usual amount of crossing-over.

Neither of these genes causes any change in the usual condition of no crossing-over in males.

An incompletely investigated case of increased crossing-over between purple and curved is apparently due, in part at least, to a dominant third-chromosome gene.

A cross-over gene, located in the third chromosome, affects that chromosome in much the same way that $C_{II\,r}$ affects the region in which it lies.

In all these cases the amount of crossing-over is changed, often markedly so. But the sequence of the genes is unchanged; and females of any one constitution give as consistent results as do "normal" females.

APPENDIX.

DETAILED DATA.

In the following tables it is to be understood that when a theoretically possible cross-over class is not set down no flies representing such a cross-over appeared in the series involved.

TABLE 26.

ONE ORIGINAL NOVA SCOTIA CHROMOSOME.

Culture.	Non-cross-overs.		Cross-overs.		Total.
	+	$b\ m_7$	b	m_7	
645	98	38	0	1	137
646	96	40	2	2	140
Total	194	78	2	3	277

Culture.	Non-cross-overs.		Cross-overs.		Total.
	+	$v_g\ s_p$	v_g	s_p	
7	55	44	0	0	99
68	75	59	2	0	136
69	72	46	2	0	120
109	61	52	0	0	113
170	32	23	0	0	55
199	156	169	0	0	325
200	161	178	0	0	339
Total	612	571	4	0	1,187

Culture.	Non-cross-overs.		Cross-overs.		Total.
	+	$c\ s_p$	c	s_p	
193	111	86	0	1	198
281	89	61	0	0	150
282	143	122	0	0	265
308	66	56	0	0	122
310	87	63	0	0	150
527	79	23	0	0	102
541	99	86	0	0	185
Total	674	497	0	1	1,172
527a[1]	109	99	0	0	208

[1] Second brood of same.

TABLE 26—continued.

Culture.	Non-cross-overs.		Cross-overs.						Total.
			1		2		1, 2		
	+	$b\ p_r\ c\ m_r$	b	$p_r\ c\ m_r$	$b\ p_r$	$c\ m_r$	p_r	$b\ c\ m_r$	
675.....	67	49	0	1	3	2	0	0	122
683.....	132	125	0	0	1	0	0	0	258
691.....	84	38	0	0	1	4	0	0	127
691a....	101	66	0	0	1	2	0	0	170
692.....	123	106	0	0	0	1	0	0	230
695.....	105	79	0	0	0	0	0	0	184
726.....	104	83	0	0	4	7	1	0	199
727.....	105	43	0	0	1	1	0	0	150
758.....	54	62	0	0	0	3	0	0	119
759.....	97	89	0	0	2	1	0	0	189
760.....	61	45	0	0	2	3	0	0	111
761.....	96	69	0	0	1	2	0	0	168
762.....	73	52	0	0	0	0	0	0	125
Total..	1,202	906	0	1	16	26	1	0	2,152
675a[1]...	163	112	0	3	0	2	0	0	280
684a[1]...	145	112	0	0	0	2	1	0	260
691b[1]...	164	124	0	0	2	2	0	0	292
692a[1]...	52	62	0	0	1	0	1	0	116
Total..	524	410	0	3	3	6	2	0	948

[1] Second broods from 675, 684, 691, and 692 above.

Culture.	Non-cross-overs.		Cross-overs.		Total.
			2		
	$S'\ b\ p_z$	+	$S'\ b$	p_z	
733.....	91	103	3	3	200
735.....	88	102	3	3	196
Total.....	179	205	6	6	396

Culture.	Non-cross-overs.		Cross-overs.						Total.
			1		2		1, 2		
	+	$b\ p_r\ c$	b	$p_r\ c$	$b\ p_r$	c	p_r	$b\ c$	
368.....	90	42	0	0	0	0	0	0	132
369.....	124	70	0	1	0	1	0	0	196
370.....	82	57	0	0	3	0	0	0	142
410.....	67	33	0	0	0	0	0	0	100
411.....	100	68	1	1	3	2	0	0	175
412.....	94	80	0	0	1	0	0	0	175
434.....	236	160	0	1	1	0	0	0	398
436.....	88	87	0	0	0	0	0	0	175
437.....	82	43	1	0	0	0	0	0	126
441.....	151	126	0	0	2	2	0	0	281
442.....	112	76	1	0	1	2	0	0	192
443.....	156	125	1	0	1	2	0	0	285
444.....	124	101	0	0	0	0	0	0	225
445.....	247	227	1	1	0	0	0	0	476

TABLE 26—continued.

Culture.	Non-cross-overs.		Cross-overs.						Total.
	+	*b* p_r *c*	1		2		1, 2		
			b	p_r *c*	*b* p_r	*c*	p_r	*b c*	
460.....	211	170	3	1	0	0	0	0	385
461.....	201	132	3	0	1	2	0	0	339
462.....	200	136	1	0	1	0	1	0	339
463.....	165	103	0	0	1	0	0	0	269
464.....	133	81	0	1	1	1	0	0	217
474.....	166	95	0	0	2	1	1	0	265
475.....	58	41	0	0	0	0	0	0	99
476.....	140	136	0	0	0	1	1	0	278
477.....	107	58	0	0	3	5	0	0	173
494.....	82	66	0	0	1	2	0	0	151
501.....	94	100	0	0	2	0	0	0	196
502.....	112	77	0	0	2	1	0	0	192
503.....	122	122	0	0	1	0	0	0	245
505.....	84	56	1	0	1	1	0	0	143
507.....	64	34	0	0	2	0	0	0	100
553.....	87	70	0	0	0	0	0	0	157
554.....	113	77	0	0	0	1	0	0	191
581.....	74	54	0	0	2	1	0	0	131
601.....	115	74	0	0	1	0	0	0	190
639.....	97	87	1	1	2	1	0	0	189
676.....	106	72	0	3	1	2	0	0	184
677.....	116	43	0	0	2	0	0	0	161
678.....	134	66	0	0	1	3	0	0	204
679.....	122	73	0	1	1	1	0	0	198
680.....	123	76	0	0	0	0	0	0	199
681.....	132	107	0	1	0	3	0	0	243
681*a*....	92	73	0	0	1	0	0	0	166
682.....	104	54	0	0	1	1	0	0	160
684.....	137	64	0	0	0	2	0	0	203
685.....	116	53	0	0	2	0	0	0	171
693.....	91	65	0	0	0	0	0	0	156
694.....	98	63	0	0	0	0	0	0	161
Total..	5,549	3,873	14	12	44	38	3	0	9,533
478[1]....	109	82	0	2	4	5	0	0	202
502*a*[1]...	113	75	0	0	0	0	0	0	188
510[1]....	142	81	0	0	1	3	1	0	228
601*a*[1]...	87	52	0	0	1	0	0	0	140
694*a*[1]...	94	93	0	0	0	1	0	0	188
Total..	545	383	0	2	6	9	1	0	946

[1] Second broods from above.

Culture.	Non-cross-overs.		Single cross-overs.					Total.
	+	*b* p_r v_g (a_r) s_p	2		3	4		
			b p_r	v_g (a_r) s_p		*b* p_r v_g (a_r)	s_p	
258.....	121	61	1	0	0	0	0	183
259.....	127	74	1	1	0	0	0	203
262.....	77	41	0	0	0	0	1	119
304.....	129	93	2	1	0	0	0	225
656.....	146	99	2	1	0	0	0	248
659.....	64	51	0	1	0	0	0	116
Total....	664	419	6	4	0	0	1	1,094

TABLE 26—continued.

	Non-cross-overs.		Cross-overs.				Total.
			2		3		
	+	$S'\ b\ p_r\ c\ s_p$	$S'\ b$	$p_r\ c\ s_p$	$S'\ b\ p_r$	$c\ s_p$	
819.....	112	110	0	0	1	0	223
819a[1].....	108	111	1	0	0	2	222

[1]Second brood from above.

SPECK END REPLACED.

Culture.	Non-cross-overs.		Cross-overs.				Total.
			2		1, 2		
	+	$b\ p_r\ v_g\ (a_r)\ s_p$	$b\ p_r$	$v_g\ (a_r)\ s_p$	p_r	$b\ v_g\ (a_r)\ s_p$	
422.....	133	144	3	2	1	0	283

Culture.	Non-cross-overs.		Cross-overs.				Total.
			1		2		
	+	$b\ p_r\ c$	b	$p_r\ c$	$b\ p_r$	c	
477....	175	97	1	0	4	1	278
492....	116	90	0	0	1	0	207
Total.	291	187	1	0	5	1	485

Culture.	Non-cross-overs.		Cross-overs.		Total.
	b	s_p	+	$b\ s_p$	
337.....	125	188	2	0	315
341.....	118	134	3	2	257
Total.	243	322	5	2	572

ONE RECONSTITUTED NOVA SCOTIA CHROMOSOME.

Culture.	Non-cross-overs.		Cross-overs.		Total.
			2		
	+	$b\ p_r\ c\ s_p$	$b\ p_r$	$c\ s_p$	
786.....	138	141	4	0	283
787.....	137	133	1	1	272
Total.	275	274	5	1	555

HETEROZYGOUS C_{II_r}.

Culture.	Non-cross-overs.		Cross-overs.		Total.
	+	$b\ p_r$	b	p_r	
352.....	142	143	8	3	296
353.....	151	142	4	10	307
Total..	293	285	12	13	603

Culture.	Non-cross-overs.		Cross-overs.		Total.
	+	$b\ c$	b	c	
468.....	72	65	10	7	154

TABLE 26—continued.

Culture.	Non-crossovers.		Crossovers.		Total.	Culture.	Non-crossovers.		Crossovers.		Total.
	b	*c*	+	*b c*			+	*b* m_r	*b*	m_r	
277.....	64	38	3	0	105	653.....	183	97	12	1	293
377.....	104	133	9	9	255	654.....	146	89	11	2	248
379.....	114	80	10	9	213	655.....	80	95	10	8	193
380.....	55	65	3	5	128						
Total..	337	316	25	23	701	Total.	409	281	33	11	734

Culture.	Non-crossovers.		Crossovers.		Total.	Culture.	Non-crossovers.		Crossovers.		Total.
	+	p c_r	p_r	*c*			+	*c* s_p	*c*	s_p	
351.....	64	69	4	2	139	226.....	102	44	0	0	146
495.....	173	156	0	0	329						
517.....	70	77	0	0	147						
Total..	307	302	4	2	615						

HETEROZYGOUS C_{II}.

Culture.	Non-crossovers.		Crossovers.						Total.
	+	*b* p_r *c*	.1		2		1, 2		
			b	p_r *c*	*b* p_r	*c*	p_r	*b c*	
318.....	136	91	7	6	2	1	0	0	243
354.....	106	105	7	4	4	0	0	0	226
355.....	103	81	7	4	1	1	0	0	197
416.....	72	42	3	6	0	0	0	1	124
417.....	109	49	8	4	3	2	0	0	175
430.....	96	77	9	4	1	4	0	0	191
431.....	136	114	4	10	6	0	0	0	270
432.....	143	111	7	8	3	2	0	0	274
433.....	80	53	2	3	0	0	0	0	138
446.....	177	179	9	6	0	1	1	0	373
448.....	58	39	1	1	1	0	0	0	100
449.....	142	124	2	2	0	0	1	0	271
450.....	156	151	6	8	1	1	0	0	323
467.....	196	167	14	11	5	1	0	1	395
469.....	205	191	11	15	4	2	1	1	430
470.....	184	124	21	18	3	3	0	0	353
471.....	178	136	10	16	2	4	0	0	346
472.....	178	147	7	10	3	0	0	0	345
473.....	145	95	2	5	6	1	0	0	254
480.....	177	104	7	2	4	3	0	0	297
496.....	132	89	7	4	1	3	1	0	237
500.....	137	122	7	8	1	1	1	0	277
511.....	51	49	2	4	2	1	0	0	109
Total..	3,096	2,440	160	159	53	31	5	3	5,947
450*a*[1]....	95	100	7	7	4	4	1	0	218
472*a*[1]....	114	102	23	14	6	3	1	0	263
Total..	209	202	30	21	10	7	2	0	481

[1] Second broods from 450 and 472 above.

TABLE 26—continued.

Culture.	Non-crossovers.		Crossovers.						Total.
			1		2		1, 2		
	$b\ p_r$	c	p_r	$b\ c$	+	$b\ p_r\ c$	b	$p_r\ c$	
686.....	114	89	6	4	4	3	0	1	221
687.....	52	49	5	3	1	2	0	0	112
707.....	48	55	3	6	0	0	1	0	113
708.....	44	62	11	2	0	2	0	0	121
709.....	53	44	1	1	1	0	0	0	100
719.....	94	67	1	7	0	0	0	0	169
721.....	108	94	8	7	0	3	0	0	220
Total.	513	460	35	30	6	10	1	1	1,056
707a[1]...	107	82	10	1	1	1	0	0	202
708a[1]...	108	122	6	2	0	1	0	1	240
Total.	215	204	16	3	1	2	0	1	442

Culture.	Non-crossovers.		Crossovers.				Total.
			1		2		
	b	$p_r\ c$	+	$b\ p_r\ c$	p_r	$b\ c$	
512.....	136	104	11	6	0	2	259
513.....	142	118	23	3	1	0	287
Total.....	278	222	34	9	1	2	546

Culture.	Non-crossovers.		Crossovers.				Total.
			1		2		
	+	$b\ p_r\ s_p$	b	$p_r\ s_p$	$b\ p_r$	s_p	
569.....	101	92	7	0	3	3	206
702.....	74	80	2	5	1	1	163
Total.....	175	172	9	5	4	4	369

Culture.	Non-crossovers.		Crossovers.						Total.
			1		2		1, 2		
	$b\ p_r$	s_p	$b\ s_p$	p_r	+	$b\ p_r\ s_p$	b	$p_r\ s_p$	
545.....	97	91	6	22	11	12	0	2	241
546.....	99	91	15	22	13	14	1	3	258
547.....	73	90	4	3	3	0	5	0	178
Total.	269	272	25	47	27	26	6	5	677
546a[2]...	106	128	26	24	17	18	3	1	323

[1] Second broods of 707 and 708 above.
[2] Second broods of 546 above.

TABLE 26—continued.

Culture.	Non-crossovers.		Crossovers.		Total.
	+	$b\ v_g\ s_p$	1		
			b	$v_g\ sp$	
648.....	85	56	11	4	156
648a[1]....	122	95	3	2	222

Culture.	Non-crossovers.		Crossovers.												Total.
			1		2		3		1, 2		1, 3		2, 3		
	S'	$b\ p_r\ s_g$	$S'\ b\ p_r\ s_g$	4	$S'\ p_r\ s_g$	b	$S'\ s_g$	$b\ p_r$	$S'\ b$	$p_r\ s_g$	$S'\ b\ p_r$	s_g	$S'\ p_r$	$b\ s_g$	
696.....	45	47	26	48	5	2	4	0	1	2	0	1	0	0	181
699.....	20	27	20	15	2	1	1	0	0	1	1	1	0	1	90
715.....	61	60	38	45	1	6	0	2	2	2	0	0	0	0	217
716.....	37	42	20	24	3	2	0	0	0	0	0	0	0	0	128
717.....	39	42	29	32	3	1	2	1	0	1	0	0	1	0	151
Total.	202	218	133	164	14	12	7	3	3	6	1	2	1	1	767
696a[2]...	63	55	50	46	1	2	1	0	0	2	0	0	0	0	220
699a[2]...	17	28	23	20	2	6	0	1	0	1	1	1	0	0	100
Total.	80	83	73	66	3	8	1	1	0	3	1	1	0	0	320

Culture.	Non-crossovers.		Crossovers.				Total.
	+	$b\ p_r\ c\ m_r$	1		2		
			b	$p_r\ c\ m_r$	$b\ p_r$	$c\ m_r$	
723.....	93	82	1	3	2	4	185
724.....	115	94	7	7	4	5	232
725.....	101	79	4	5	5	1	195
763.....	64	72	9	3	3	2	153
Total.....	373	327	21	18	14	12	765

Culture.	Non-crossovers.		Crossovers.						Total.
	s_g	$b\ p_r\ c$	1		2		1, 2		
			$b\ s_g$	$p_r\ c$	$b\ p_r\ s_g$	c	$p_r\ s_g$	b_c	
742.....	142	150	8	8	1	2	0	0	311
743.....	136	143	4	6	1	1	0	0	291
747.....	71	56	8	6	0	1	0	0	142
749.....	112	133	10	9	3	3	1	0	271
Total.....	461	482	30	29	5	7	1	0	1,015

[1] Second brood of above. [2] Second broods of 696 and 699, above.

TABLE 26—continued.

Culture.	Non-crossovers.		Crossovers.							
			1		2		3		4	
	$S'\,b\,s_p$	$p_r\,c$	$S'\,p_r\,c$	$b\,s_p$	$S'\,b\,p_r{}^c$	s_p	$S'\,b\,c$	$p_r\,s_p$	$S'\,b$	$p_r\,c\,s_p$
796	82	69	71	73	5	11	1	0	0	0
797	68	52	42	42	4	5	0	2	0	0
798	82	56	49	54	13	8	3	1	0	1
799	71	54	36	59	7	7	1	3	0	0
800	73	70	60	66	6	9	2	1	0	0
801	56	72	53	52	6	5	0	3	0	0
Total	432	373	311	346	41	45	7	10	0	1
796a[1]	90	68	62	64	4	6	6	2	0	0
797a[1]	68	65	45	53	7	8	3	2	0	0
798a[1]	80	64	30	38	0	7	1	2	0	0
Total	238	197	137	155	11	21	10	6	0	0

Culture.	Crossovers—Continued.												Total.
	1, 2		1, 3		2, 3		2, 4		3, 4		1, 2, 3		
	$S'\,s_p$	$b\,p_r\,c$	$S'\,p_r\,s_p$	$b\,c$	$S'\,b\,p_r\,s_p$	c	$S'\,b\,p_r\,c\,s_p$	+	$S'\,b\,c\,s_p$	p_r	$S'\,c$	$b\,p_r\,s_p$	
796	5	5	1	0	1	1	0	0	0	1	0	0	325
797	3	2	1	0	0	0	0	0	0	0	0	0	221
798	0	2	0	0	0	0	0	0	0	0	0	0	269
799	1	6	0	1	0	0	0	0	0	0	2	0	248
800	3	4	0	2	0	0	0	0	0	0	0	0	296
801	0	1	1	5	0	0	1	0	0	1	0	0	256
Total	12	20	3	8	1	1	1	0	0	1	2	0	1,615
796a[1]	1	0	0	1	2	0	0	0	0	0	0	1	307
797a[1]	1	1	4	0	0	1	0	0	0	0	1	0	259
798a[1]	4	2	0	0	0	0	0	0	0	0	0	0	228
Total	6	3	4	1	2	1	0	0	0	0	1	1	794

[1] Second broods from 796, 797, and 798 above.

Culture.	Non-crossovers.		Crossovers.		Total.
			2		
	$S'\,b$	c	$S'\,b\,c$	+	
776	52	54	22	16	144
795	51	43	8	8	110
Total	103	97	30	24	254
795a[1]	51	45	6	5	107

[1] Second brood of 795, above.

TABLE 26—continued.

Culture.	Non-crossovers.		Crossovers.				Total.	Culture.	Non-crossovers.		Crossovers.						Total.
			1		2						1		2		1, 2		
	+	$b\ p_r\ v_g\ (a_r)\ s_g$	b	$p_r\ v_g\ (a_r)\ s_g$	$b\ p_r$	$v_g\ (a_r)\ s_g$			+	$b\ p_r\ s_g$	b	$p_r\ s_g$	$b\ p_r$	s_g	p_r	$b\ s_g$	
404.....	114	84	7	3	2	0	210	785.....	64	74	0	1	54	55	1	0	249

Culture.	Non-crossovers.		Crossovers.						Total.
			2		3		2, 3		
	$b\ p_r\ s_g$	c	$b\ p_r\ c$	s_g	$b\ p_r$	$c\ s_g$	+	$b\ p_r\ c\ s_g$	
794.....	41	50	17	18	46	22	1	5	200
794a[1]...	76	61	19	17	47	35	6	0	261

[1] Second brood of 794, above.

$$\frac{C_{II\,2}}{C_{II\,7}}$$

Culture.	Non-crossovers.		Crossovers.						Total.
			2		3		4		
	$S'\ b\ p_r\ s_g$	c	$S'\ b\ c$	$p_r\ s_g$	$S'\ b\ p_r$	$c\ s_g$	$S'\ b\ p_r$	$c\ s_g$	
789.....	112	116	0	0	5	5	0	0	238
790.....	95	124	0	0	1	1	1	0	222
791.....	107	105	1	0	6	6	0	0	225
Total.	314	345	1	0	12	12	1	0	685
789a[1]...	141	117	0	0	3	4	0	1	266
791a[1]...	115	124	0	0	1	3	0	0	243
Total.	256	241	0	0	4	7	0	1	509

[1] Second broods of 789 and 791 above.

Culture.	Non-crossovers.		Crossovers.		Total.	Culture.	Non-crossovers.		Crossovers.				Total.
			2						1		2		
	$b\ p_r$	$c\ m_r$	+	$b\ p_r\ c\ m_r$			$b\ p_r$	c	p_r	$b\ c$	+	$b\ p_r\ c$	
713.....	95	103	6	3	207	754.....	48	58	1	1	2	0	110
752.....	126	131	1	1	259	778.....	61	59	0	0	6	2	128
753.....	94	82	0	1	177	Total.	109	117	1	1	8	2	238
Total..	315	316	7	5	643								

TABLE 26—continued.

$\frac{C_{II\,2}\ C_{II\,7}}{C_{II\,7}}$

Culture.	Non-crossovers.		Crossovers.								Total.
			1		3		1, 3		1, 2, 3		
	$S'\,b\,p_r\,s_p$	+	S'	$b\,p_r\,s_p$	$S'\,b\,p_r$	s_p	$S'\,s_p$	$b\,p_r$	$S'\,p_r$	$b\,s_p$	
736.....	54	47	1	0	39	44	0	0	1	0	186
738.....	78	71	0	0	61	66	0	0	1	0	277
739.....	37	35	0	2	41	36	0	0	0	0	151
740.....	39	50	0	0	32	27	0	0	0	0	148
779.....	73	73	0	0	68	72	0	0	0	0	286
780.....	31	49	0	0	29	30	0	0	0	0	139
782.....	45	73	0	0	40	52	0	0	0	0	210
783.....	59	60	0	0	56	64	1	0	0	0	240
784.....	84	89	0	0	91	94	0	0	0	0	358
Total.....	500	547	1	2	457	485	1	0	2	0	1,995

Culture.	Non-crossovers.		Crossovers.				Total.
			2		1, 2		
	+	$b\,p_r\,s_p$	$b\,p_r$	s_p	p_r	$b\,s_p$	
660.....	27	31	18	24	0	1	101
670.....	55	41	49	47	0	0	192
Total.	82	72	67	71	0	1	293

Culture.	Non-crossovers.		Crossovers.		Total.
	b	s_p	+	$b\,s_p$	
340.....	72	72	70	49	263

$\frac{C_{II\,7}}{C_{II\,7}}$

Culture.	Non-crossovers.		Crossovers.														Total.
			1		2		3		1, 2		1, 3		2, 3		1, 2, 3		
	$S'\,b$	$p_r\,s_p$	$S'\,p_r\,s_p$	b	$S'\,b\,p_r\,s_p$	+	$S'\,b\,s_p$	p_r	S'	$b\,p_r\,s_p$	$S'\,p_r$	$b\,s_p$	$S'\,b\,p_r$	s_p	$S'\,s_p$	$b\,p_r$	
885....	40	43	26	30	3	2	30	27	0	0	23	16	1	2	0	0	243
886....	44	47	30	31	1	0	37	39	0	1	22	18	4	1	1	2	276
887....	17	27	14	14	1	0	15	29	0	1	7	17	1	0	0	1	144
888....	54	31	24	23	3	6	45	20	0	0	32	21	2	1	0	0	262
Total.	155	148	94	98	8	8	127	115	0	2	84	72	8	4	1	3	927

Culture.	Non-crossovers.		Crossovers.						Total.
			1		2		1, 2		
	$b\ p_r$	s_p	$b\,s_p$	p_r	+	$b\,p_r\,s_p$	b	$p_r\,s_p$	
745.....	75	90	2	1	41	30	1	3	243
748.....	79	51	4	2	43	47	0	2	228
Total..	154	141	6	3	84	77	1	5	471

Culture.	Non-crossovers.		Crossovers.		Total.
	b	s_p	+	$b\,s_p$	
623.....	41	36	39	35	151

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IV.

A DEMONSTRATION OF GENES MODIFYING THE CHARACTER "NOTCH."

By T. H. Morgan.

A DEMONSTRATION OF GENES MODIFYING THE CHARACTER "NOTCH."

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Two main topics are dealt with in the following pages from the standpoint of the experimental results obtained. One of them concerns the demonstration of modifying genes that were involved in the results of a selection experiment. The other topic is a discussion of the possibility of contamination of genes as a method that has been appealed to as an influence vitiating the regularity of Mendelian phenomena.

The claim of the Mendelians that genes have been found to be stable in successive generations wherever a critical test of them was made has been challenged both on the grounds of empiric observation and on the more sentimental grounds that such hard and fast rules do not apply to living things which are rather to be thought of as variable quantities. In the following pages an account is given of a character that changed in the course of selection and a demonstration that the result was due to a modifying gene and not to contamination between the notch gene and its normal allelomorph, despite the fact that an exceptional opportunity was given to contaminate the gene, if contamination is a possible process.

In 1915, Dexter described a mutant type of *Drosophila* called Notch or "perfect Notch," and made out the main points in the heredity of the character. The gene is sex-linked, and dominant for the serration that it produces in the wings, but recessive in its lethal effect. Since the gene is carried by the X chromosome, any male that gets a chromosome with this gene dies, while the female that has another X carrying the normal allelomorph lives and shows the notch at the end of her wings (fig. 91). Since no male that has the notch gene can live, it is not feasible to determine whether a female containing two lethal bearing X's would also die.[1] Every heterozygous Notch female gives twice as many daughters as sons, because, as stated, half of the sons die, namely, those that get the lethal-bearing X^n. The scheme is as follows:

$$\begin{array}{c} X - X^n \text{ eggs} \\ X - Y \text{ sperm} \\ \hline XX - XX^n - XY - X^nY \text{ (dies)} \\ \text{♀} \quad \text{♀} \quad \text{♂} \quad \text{♂} \end{array}$$

[1] Unless an XX egg, arising through "equational non-disjunction," were fertilised by a Y sperm and lived. No such females have appeared. They would have no regular sons.

Half of the daughters are normal, half are heterozygous Notch. The normal daughters and normal sons never transmit the Notch gene, which, therefore, never gets into the male line or into the line of normal daughters.

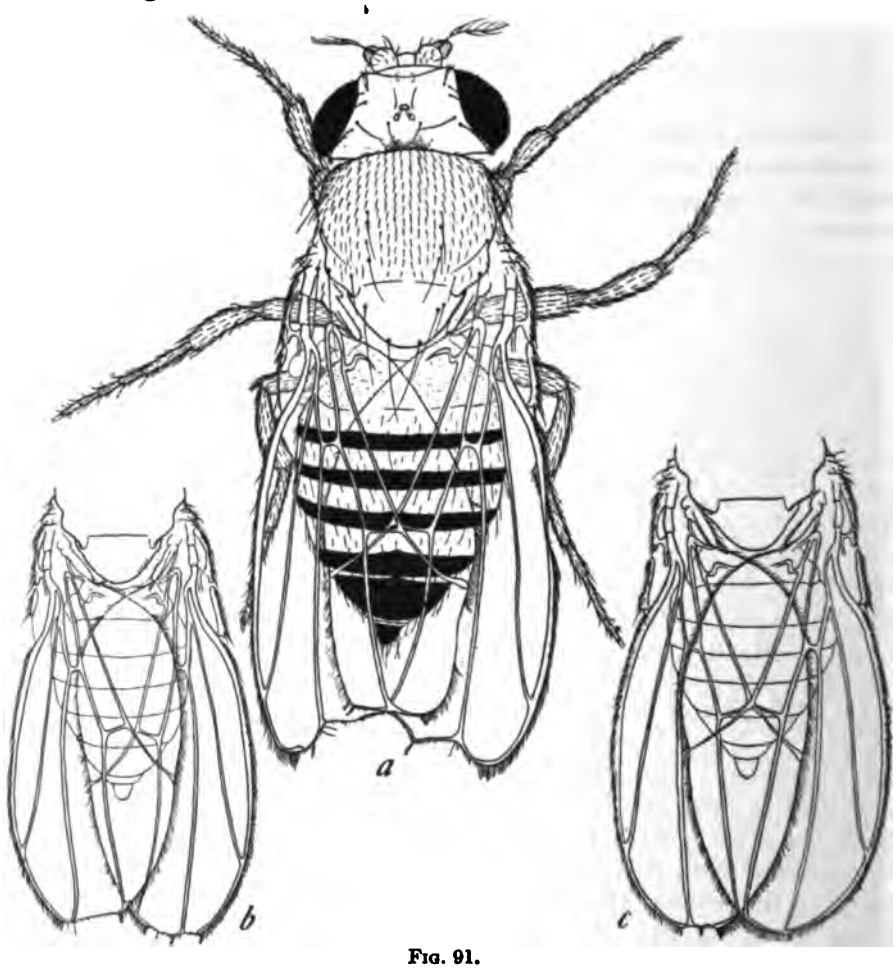


FIG. 91.

Dexter obtained his mutant in a cross between beaded and wild. The Notch that I used arose independently in descendants of vestigial flies, in which stock the factor may have already existed. This mutant has, however, originated several times in other cultures in the laboratory. It is by no means one of the rarer mutations.

VARIATION OF NOTCH.

The most conspicuous character of the female heterozygous for Notch is the serration at the end of the wings (fig. 91) caused by the absence of the marginal bristles and generally accompanied by a slight

concavity of the edge. The range of variation of the notching is very wide. That the limit of variability overlaps in one direction the normal wing is certain, for amongst the daughters without notching occasionally one is found half of whose daughters are notched. The not unusual occurrence of a fly with one entire wing and one with notching (fig. 91, *c*) indicates that the range of variation includes normal wings. The low *productivity* of the Notch female appears to be an incidental effect of the Notch factor, because the normal sisters of the same stock are, whenever tested, much better producers. The *viability* of the Notch females is fairly good, but they appear to run behind their normal sisters in nearly all cultures. Change in the viability will be discussed later.

THE PROBLEM.

Throughout the older literature dealing with selection, the idea that the grade of any character shown by animals or plants is a criterion of the condition of the genetic factor or gene responsible for the character continually recurs, and the same idea appears occasionally in more recent times, despite Johanssen's analysis showing the inconsequence of such an argument, and despite the accumulated demonstrations that the production of a given character depends on the environment and on internal modifying genes, as well as on the principal gene itself. The wide range of variability of the notching, the fact that the females genetically Notch may be identified by the 2 to 1 sex-ratio in the offspring, even when the wings themselves have somatically the normal margin, as well as the fact that it is a dominant, and therefore any alteration in the gene may be tested directly by outbreeding; the fact that linkage relations made it possible to identify any changes that might follow selection in the individuals that were Notch, although with normal wings; all these made Notch excellent material on which to put to actual test some of the older as well as current views concerning the nature of Mendelian factors and the influence of selection. In each generation several (usually 2 to 10) virgin, notched-winged females (of the derived type) were picked out and put into a new bottle with one to 10 males. Occasionally pairs were used and then mass selection followed in the next generations. This prodecure is not unlike the rough procedure formerly practised by the breeder, but is not, of course, to be recommended for a thorough understanding of the changes that are taking place during the selection period. Moreover, by such a method the end result is attained only after a long time, whereas the results here described could probably have been reached in two or three generations; for, as the duplicate experiments show, the modifying gene for "slight notch" did not arise in the course of the experiment, but was present in some flies of the stock at the beginning.

On the other hand, had the sequel shown that the results were due to a number of factors present at the beginning, the mass-culture method would have offered a better chance of collecting the different modifiers in the same strain. The object of the selection process here practised was, however, to produce by a rough method results of the kind familiar to the breeder, and then to show, by the refined tests that the *Drosophila* work has made possible, what had been done to the original stock.

CONDITION OF STOCK BEFORE SELECTION.

In table 1 there are records of the offspring of 11 pairs of Notch females by normal males. The totals give 577 Notch females, 608 normal females, 613 normal males. It is clear that the viability of the Notch females compares favorably with that of the normal females. Very few of the Notch flies could have had normal wings when this class comes so near to the realization of their expected numbers. However, there were other females that had the same origin in which the ratios amongst the offspring were strikingly different. These are given here in table 2.

TABLE 1.

Ref.	Notch.	Normal ♀.	Normal ♂.	Ref.	Notch.	Normal ♀.	Normal ♂.
PT.....	40	49	35	50–1.....	45	51	60
PN.....	9	8	9	PN.....	42	52	55
Pn.....	29	21	32	SS.....	37	46	41
50–1.....	40	40	42	50–1.....	205	219	212
PN.....	42	46	44				
PN.....	36	35	27	Total...	577	608	613
PN.....	52	40	46				

TABLE 2.

Ref.	Notch.	Normal ♀.	Normal ♂.	Ref.	Notch.	Normal ♀.	Normal ♂
U.....	25	91	36	SSG.....	89	137	114
Pu.....	47	77	65	SSO.....	49	115	99
SS.....	35	126	147	DO.....	52	170	106
SS.....	39	122	97				
EVN.....	75	182	172	Total..	409	1,020	836

In these 8 sets the Notch females are not half as numerous as the males and less than half as numerous as the normal females. The normal females are greatly in excess of the males. If we suppose that here a considerable number of the Notch females have normal wings—as was actually shown to be the case later in the offspring of some of these sets—the discrepancies between tables 1 and 2 may be accounted for. Thus, if we add the two classes of females (1429) and divide by

2 to give the expected number of Notch females (viz, 714), the results would mean that about 300 of the Notch females had varied into the normal class of females.

We may make the comparison in another way. If the number of the males be taken as the measure of each class of females, there will be over 400 too few Notch females, and about 200 too many normal females.

It was the offspring of some of these lots, viz, the SS lots, that later furnished the materials for selection (SSO, SSO 1, etc.). If the above interpretation be accepted as plausible, then at the beginning of the experiment either different genes for Notch were present or modifying genes were there. The later tests proved the presence of a modifying gene, but since this is not sex-linked, it may have been present in certain of the females or males either in heterozygous or homozygous condition, hence, until the stock could be made homozygous for this gene, random selection would be expected to give for some time variable results.

SELECTION OF FEMALES HAVING NOTCH IN ONE WING ONLY.

If the somatic characters were an index of the condition of the differentiating factor for a character, it would appear that those flies in which the character appeared in only one wing should indicate a change towards the phenotypic normal end of the variation curve. Hence by selecting in successive generations as parents those flies that had the character only in one wing, and amongst these only those in which it was developed to the slightest visible extent, then one might expect to bring about a change, but of course this would be equally true whether the selection was based on a changing factor or on the more frequent presence of one or more modifying factors. An experiment of this sort was begun in the third generation after SSO (viz, in SSO 112)and continued through 11 generations, with the result shown in the table 3. In the first column are given the flies in which both wings are notched, in the second the flies with a notch in only one wing, in the third the females with normal wings, and the fourth the males. I have indicated by the star (*) those records in which it appears that a considerable portion of the potential Notch females fall into the phenotypic normal class as shown by the excess of normal females and the deficiency of Notched females over the number of the males. This change is noticeable in the sixth to the eleventh generation. In the last 4 generations this relation holds for all the cultures, with two exceptions only in the eighth generation. It is probable, therefore, that at this time the full force of selection has been accomplished and there is nothing to indicate that unless some new sort of change were to occur, selection would accomplish anything further after the ninth generation.

SELECTION OF SOMATICALLY NORMAL WINGED FEMALES THAT ARE GENETICALLY NOTCHED FEMALES.

At the beginning of the work a few lines were run with eosin ruby males which were bred to the Notch females, but the history of these

TABLE 3.—*SS Set.*

Gen.	Both Notch ♀.	One Notch ♀.	Normal ♀.	Normal ♂.
1	17	14	88	*63
1	15	2	18	18
1	8	7	27	29
1	23	19	136	*109
Total	63	42	269	219
2	9	4	19	4
2	9	4	19	4
2	38	14	55	64
Total	56	22	93	72
3	5	1	17	7
3	12	9	28	27
3	11	4	38	*40
3	16	12	49	50
Total	44	26	132	124
4	6	4	42	37
4	7	4	13	14
4	15	2	29	25
4	14	17	29	25
Total	42	17	113	101
5	13	7	24	14
5	3	10	35	26
5	16	4	38	*22
5	4	1	5	5
5	19	3	45	*33
5	4	5	17	17
5	11	13	52	*24
5	3	2	6	2
5	6	4	22	18
Total	79	49	254	161
6	9	4	11	8
6	3	8	32	18
6	6	20	127	*41
6	3	3	14	13
6	6	14	102	*61
6	5	7	40	*20
6	1	10	39	*19
Total	33	66	365	180
7	0	7	35	*9
7	3	8	19	15
7	0	4	19	21
7	0	7	14	14
7	4	5	15	12
7	1	15	76	*29
7	7	7	12	9
7	0	7.	14	14
7	6	11	31	29
7	18	12	228	*167
7	3	7	28	*19
7	1	1	29	*11
Total	43	91	520	349
8	2	15	21	22
8	11	10	29	20
8	7	12	72	*37
8	5	13	104	*72
8	11	5	24	*14
8	1	19	130	*68
Total	37	74	380	233
9	1	5	45	*30
9	2	9	56	*33
9	8	23	161	*75
9	2	8	38	*18
9	4	23	105	*73
Total	17	68	405	229
10	10	6	45	*19
10	16	13	55	*43
10	15	27	149	*88
10	0	4	24	*24
10	1	11	55	*26
10	5	2	118	*64
10	3	12	65	*42
10	3	8	96	*42
10	0	1	21	* 9
10	3	12	85	*42
Total	56	96	713	393
11	6	19	107	*42
11	10	10	39	*19
11	8	8	63	*49
11	1	6	50	*27
Total	25	43	259	137

lines is not clearly separable now from those recorded in the last section. There is, moreover, the possibility that during these early experiments, stock males of eosin ruby may have been introduced at one stage. That these conditions have not affected seriously the condition of the selected stock as a whole is shown by table 4, where the number of normal females belonging to the potential Notch class is as high in most cases as in the middle and latter parts of the preceding table.

By introducing into the experiments the two genes eosin and ruby, it is a very simple matter to identify potentially Notch females from the other females with normal wings. Selecting the former makes it possible to carry on the experiment by breeding in every generation from those females that carry the factor for Notch, but do not show a notch in the wing. In other words, if the expression of a character (its phenotype) is a measure of the major factor that produces it, we should expect that an extreme selection of this kind would be an excellent way of fixing the factor altered by selection.

The location of the Notch factor had shown that it lies in a region of the X chromosome (fig. 92), 2.8 units from the arbitrary zero-point yellow. Eosin lies 1.5 units and ruby lies 7.3 units from yellow. The distance between eosin and ruby is therefore a distance so short that double crossing-over never takes place within it. If, then, we use a male whose sex-chromosome contains the factors for eosin and for ruby, and a Notch female having red eyes (*i. e.*, the normal allelomorphs of eosin and of ruby) the gene for Notch in one X of the daughters will be located in a position between the eosin ruby genes present in the opposite chromosome of the same daughter, as seen in figure 92.

Now, as said above, it would necessitate double crossing-over to get the Notch gene in between the eosin and ruby genes, or, in other words, double crossing-over must take place within these limits to produce a Notch female with eosin ruby eyes. Of the many thousands of females obtained in the course of the experiment, not a single double cross-over of this kind has been observed.

Single cross-overs have, however, been recorded in the expected numbers. Thus eosin and Notch females, and eosin as well as ruby males have appeared. It would of course be possible to obtain a Notch fly with eosin ruby eyes by first getting a single cross-over of eosin Notch and then after mating such a female to an eosin ruby male some daughters, in which a cross-over between Notch and ruby would result, having eosin Notch ruby in the chromosome. Such a female bred to an eosin ruby male would give some daughters of the desired class. As there was no need in my work for such females, I have not taken the trouble to make them.

Turning to table 4, we see that nothing further resulted from selecting the potentially normal females through about 5 more generations. By potentially normal females I mean that females with red eyes and

without Notch in the wings were selected. All red-eyed females must carry the Notch, whether they show the character or not, as has been explained. At the end of the experiment the relation between the Notch and normal (of two kinds) females was about the same as that after a few generations.

TABLE 4.—*SS–162 Set.*

Gen.	Both wings Notch ♀.	One wing Notch ♀.	Normal ♀.	Eosin ruby ♀.	Eosin ruby ♂.	Eosin Notch ♀.	Ruby ♀.	Eosin ♀.	Notch ruby ♀.	Ruby ♂.	Eosin ♂.	Normal ♂.
8.....	3	+ 1	4	11	6	.....	.....	.....	.....	.....	.....	
9.....	12	+ 18	25	53	56	+1	.....	.....	.....	.....	3	.....
10.....	12	+ 22	18	75	69	.....	.....	1	.....	1	6	.....
10.....	6	+ 1	3	13	14	1	.....	2	.....	1	.....	.....
10.....	4	+ 6	12	30	25	1+1	.....	2	.....	.....	1	.....
10.....	12	+ 10	14	72	60	1	.....	8	.....	.....	.....	.....
10.....	12	+ 24	27	85	76	.....	.....	8	.....	.....	7	.....
10.....	22	+ 29	10	53	52	.....	.....	4	.....	.....	.....	.....
10.....	3	+ 10	9	17	16	1	.....	.....	.....	.....	.....	.....
10.....	6	+ 7	7	92	74	1	.....	2	.....	.....	2	.....
10.....	13	+ 24	23	68	58	3	.....	1	.....	.....	.....	.....
10.....	2	+ 6	12	40	28	1+1	.....	2	.....	.....	2	.....
Total....	92	+139	135	545	472	2+9	.....	30	.....	2	18	.....
11.....	1	+ 6	12	24	25	.....	.....	1	.....	.....	1	.....
11.....	8	+ 14	16	63	42	.....	.....	3	.....	.....	.....	.....
11.....	13	+ 24	43	126	96	.....	.....	.....	.....	.....	2	.....
11.....	2	+ 9	9	15	8	.....	.....	6	.....	.....	.....	.....
11.....	6	+ 15	17	49	26	2	.....	.....	.....	1	9	.....
11.....	6	+ 13	30	64	68	1	1	.....	.....	1	1	.....
11.....	20	+ 28	31	89	92	.....	.....	2	.....	.....	2	.....
11.....	1	+ 7	2	11	7	.....	.....	.....	.....	.....	.....	.....
11.....	7	+ 22	22	50	53	1	.....	.....	.....	.....	1	.....
11.....	9	+ 5	18	27	18	.....	.....	2	.....	.....	.....	.....
11.....	3	+ 4	12	23	26	.....	.....	.....	.....	.....	1	.....
Total....	76	+147	212	541	461	4	1	14	.....	2	17	.....
12.....	15	+ 46	48	107	100	2	.....	2	.....	2	3	.....
12.....	17	+ 9	11	24	26	.....	.....	1	.....	1	3	.....
12.....	4	+ 15	62	83	79	.....	.....	5	.....	2	4	.....
12.....	2	+ 2	34	44	36	.....	.....	1	.....	.....	1	.....
12.....	7	+ 4	18	36	44	.....	.....	5	.....	.....	1	.....
12.....	6	+ 7	21	32	24	.....	.....	4	.....	.....	1	.....
12.....	16	+ 34	53	131	155	3+2	3	3	.....	1	.....	.....
12.....	6	+ 22	31	63	75	.....	.....	1	.....	.....	3	.....
12.....	2	+ 2	17	21	20	.....	.....	.....	.....	1	.....	.....
12.....	16	+ 27	54	109	98	1	.....	10	.....	.....	1	.....
12.....	6	+ 8	12	38	28	.....	.....	.....	.....	.....	3	.....
12.....	7	+ 4	18	36	44	.....	.....	5	.....	.....	1	.....
12.....	19	+ 50	87	178	137	2	.....	6	1	.....	.....	.....
12.....	6	+ 1	5	36	25	.....	.....	2	.....	.....	1	.....
12.....	1	+ 11	27	22	22	2	.....	2	.....	.....	.....	.....
12.....	7	+ 13	23	55	64	.....	.....	2	.....	.....	5	.....
12.....	13	+ 11	22	68	58	.....	.....	.....	.....	.....	1	.....
12.....	5	+ 6	14	17	16	.....	.....	1	.....	.....	.....	.....
Total....	155	+272	557	1,000	1,051	3+9	3	50	1	7	28	.....

TABLE 4.—*SS–162 Set*—continued.

Gen.	Both wings Notch ♀.	One wing Notch ♀.	Normal ♀.	Eosin ruby ♀.	Eosin ruby ♂.	Eosin Notch ♀.	Ruby ♀.	Eosin ♀.	Notch ruby ♀.	Ruby ♂.	Eosin ♂.	Normal ♂.
13.....	2	+ 16	8	10	14	.....	.....	.....	.....	.....	.....	.....
13.....	1	+ 6	19	20	14	.....	1	1	.....	.....	1	.....
13.....	5	+ 15	21	38	37	2	.....	2	.....	.....	.....	.....
13.....	10	+ 14	79	131	111	1	.....	.....	.....	1	3	.....
13.....	6	+ 41	127	131	121	1+2	.....	3	.....	1	.....	.....
13.....	6	+ 15	31	51	34	1	.....	.....	.....	.....	3	.....
13.....	4	+ 11	14	31	22	.....	.....	.....	.....	.....	2	.....
13.....	7	+ 23	36	73	77	.....	.....	2	.....	.....	1	.....
13.....	2	+ 13	44	62	74	1	.....	1	.....	.....	.....	.....
13.....	1	+ 3	21	24	25	.....	.....	.....	.....	.....	6	.....
13.....	13	+ 42	114	169	130	3	.....	13	.....	1	.....	.....
Total....	57	+199	514	740	659	1+10	1	22	.....	3	16	.....
14.....	3	+ 13	34	50	40	.....	.....	.....	.....	.....	3	.....
14.....	7	+ 14	28	48	43	+1	.....	1	.....	.....	1	.....
14.....	2	+ 8	17	41	35	.....	.....	1	.....	.....	.....	.....
14.....	11	.....	24	72	55	.....	.....	3	.....	.....	1	.....
14.....	6	+ 9	8	17	12	.....	.....	.....	.....	.....	1	.....
14.....	13	+ 24	23	68	58	+3	.....	1	.....	.....	2	.....
14.....	2	+ 24	54	84	89	.....	.....	1	.....	1	1	.....
14.....	4	+ 9	12	20	19	.....	.....	7	.....	.....	1	.....
14.....	1	+ 9	6	14	19	.....	.....	1	.....	.....	1	.....
14.....	23	+ 43	62	136	120	.....	.....	6	.....	.....	5	.....
14.....	11	+ 26	37	57	58	1	.....	3	1	.....	1	.....
14.....	2	+ 8	17	36	38	1+2	.....	3	.....	.....	2	.....
14.....	2	+ 17	54	102	84	2	.....	.....	1	.....	1	.....
14.....	18	+ 39	62	126	120	.....	.....	6	.....	.....	9	.....
Total....	105	+243	448	871	790	1+9	.....	33	2	1	29	.....
15.....	20	− 44	21	77	79	.....	.....	5	.....	2	3	.....
15.....	11	− 28	25	72	76	.....	.....	10	.....	.....	3	.....
15.....	3	+ 8	23	69	63	.....	.....	10	.....	.....	3	.....
15.....	2	+ 3	5	12	4	+1	.....	1	.....	.....	.....	.....
15.....	0	+ 6	30	23	11	.....	.....	.....	.....	.....	.....	.....
15.....	0	+ 6	30	23	12	.....	.....	.....	.....	.....	.....	.....
15.....	20	+ 35	21	77	80	6	.....	.....	.....	2	3	.....
15.....	34	+ 30	51	127	102	1	.....	3	.....	.....	11	.....
15.....	5	+ 15	64	95	64	.....	.....	6	.....	.....	4	.....
15.....	.....	+ 4	33	25	33	.....	.....	1	.....	.....	1	.....
15.....	3	+ 4	6	26	12	+1	.....	.....	.....	.....	1	.....
15.....	.....	+ 1	12	27	12	.....	.....	.....	.....	.....	.....	.....
15.....	.....	.....	3	8	8	.....	.....	.....	.....	.....	1	.....
15.....	1	+ 3	21	54	39	.....	.....	.....	.....	.....	2	.....
Total....	99	+187	345	715	493	+9	.....	36	.....	4	32	.....
16.....	6	+ 37	46	74	65	.....	.....	7	.....	.....	4	.....
16.....	2	+ 17	58	102	86	.....	.....	2	.....	.....	2	.....
16.....	2	+ 13	61	77	71	1+1	.....	6	.....	1	12	.....
16.....	0	+ 3	6	10	9	.....	.....	.....	.....	.....	.....	.....
16.....	7	+ 32	34	83	85	1	.....	11	.....	1	1	.....
16.....	6	+ 22	117	144	138	1	1	1	.....	.....	2	.....
16.....	.....	+ 3	4	10	5	.....	.....	1	.....	.....	2	.....
16.....	2	+ 2	4	24	25	.....	.....	7	.....	.....	1	.....
Total....	25	+129	330	524	484	1+3	1	35	.....	2	24	.....

TABLE 4.—*SS-162 Set*—continued.

Gen.	Both wings Notch ♀.	One wing Notch ♀.	Normal ♀.	Eosin ruby ♀.	Eosin ruby ♂.	Eosin Notch ♀.	Ruby ♀.	Eosin ♀.	Notch ruby ♀.	Ruby ♂.	Eosin ♂.	Normal ♂
17.....	17	+ 13	24	72	71	+2	.....	8	.....	.....	9	.....
17.....	3	+ 3	6	18	15	.....	.....	1	.....	.....	.....	.....
17.....	1	+ 11	21	31	27	.....	.....	2	.....	.....	.....	.....
17.....	22	+ 24	119	160	171	1+1	1	7	.....	2	4	.....
17.....	12	+ 20	31	55	50	.....	.....	.....	.....	.....	1	.....
17.....	5	+ 6	4	11	10	1	.....	5	.....	.....	1	.....
Total....	53	+ 77	205	297	344	1+4	1	23	.....	2	15	.....
18.....	3	+ 3	12	20	16	.....	.....	.....	.....	.....	.....	.....
18.....	.....	+ 5	30	47	24	.....	.....	1	.....	.....	.....	.....
18.....	15	+ 35	75	109	95	3	.....	8	.....	2	1	.....
18.....	8	+ 28	36	106	113	1	.....	10	.....	1	6	.....
18.....	13	+ 33	51	110	85	.....	.....	7	.....	.....	3	.....
18.....	14	+ 29	26	65	69	1	.....	.....	.....	1	2	.....
18.....	6	+ 13	20	77	59	.....	.....	6	.....	.....	1	.....
18.....	24	+ 48	103	194	217	1	1	9	.....	.....	9	.....
18.....	.....	+ 2	11	20	18	.....	.....	.....	.....	.....	1	.....
18.....	1	+ 9	15	25	28	.....	.....	.....	.....	.....	.....	.....
18.....	.....	+ 1	6	7	2	.....	.....	.....	.....	.....	.....	.....
Total....	84	+206	385	780	726	6	1	41	.....	4	23	.....
19.....	.....	+ 5	18	17	21	+1	.....	1	.....	.....	1	.....
19.....	1	+ 4	18	45	26	.....	.....	2	.....	.....	1	.....
19.....	.....	+ 2	15	14	12	1	.....	2	.....	.....	.....	.....
19.....	1	+ 5	6	15	25	.....	.....	1	.....	.....	1	.....
19.....	1	.....	5	11	8	.....	.....	1	.....	.....	.....	.....
19.....	.....	+ 2	20	28	33	.....	.....	1	.....	.....	.....	.....
19.....	.....	+ 3	21	25	24	.....	.....	.....	.....	.....	1	.....
19.....	5	+ 18	36	65	64	.....	.....	1	.....	.....	2	.....
19.....	11	+ 7	6	45	34	1	.....	3	2	.....	.....	.....
19.....	2	+ 25	30	135	144	.....	.....	4	.....	.....	5	.....
Total....	21	+ 71	175	400	391	+3	.....	16	2	.....	11	.....
20.....	2	+ 2	11	51	37	+1	.....	7	.....	.....	2	.....
20.....	3	+ 1	7	8	7	.....	.....	.....	.....	.....	.....	.....
20.....	6	+ 5	14	43	43	2	1	2	.....	.....	2	.....
20.....	7	+ 18	42	59	61	1	.....	2	.....	1	4	.....
20.....	2	+ 20	28	62	39	.....	.....	3	.....	.....	2	.....
20.....	.....	+ 3	14	26	9	1	.....	1	.....	.....	2	.....
Total....	20	+ 49	116	249	196	+5	.....	15	.....	1	12	.....
21.....	4	+ 6	15	28	25	.....	.....	.....	.....	.....	1	.....
21.....	2	+ 18	14	31	33	1	.....	2	.....	.....	1	.....
21.....	2	+ 8	35	74	71	.....	.....	1	.....	1	4	.....
21.....	1	+ 8	17	45	50	.....	.....	3	.....	.....	.....	.....
Total....	9	+ 40	81	178	179	1	.....	6	.....	1	6	.....
22.....	21	+ 25	27	68	64	1	.....	5	.....	.....	1	.....
23.....	.....	+ 3	13	24	26	.....	.....	.....	.....	.....	.....	.....
23.....	8	+ 17	17	51	55	.....	.....	8	.....	.....	1	.....
Total....	8	+ 20	30	175	81	.....	.....	8	.....	.....	1	.....
24.....	1	+ 5	12	8	11	.....	.....	1	.....	.....	1	.....
24.....	4	+ 16	26	47	36	.....	.....	1	.....	.....	3	.....
Total....	5	+ 21	38	55	47	.....	.....	2	.....	.....	4	.....

DUPLICATE SELECTION EXPERIMENT.

At the time when the former series began another set (X 6 set) was started and kept apart from the former. From the third to the ninth generation, as shown in table 5, females that had Notch in only one

TABLE 5.—*X 6 Set.*

Gen.	Both wings Notch ♀.	One wing Notch ♀.	Normal ♀	Eosin ruby ♀.	Eosin ruby ♂.	Eosin Notch ♀.	Ruby ♀.	Eosin ♀.	Notch ruby ♀.	Ruby ♂.	Eosin ♂.	Normal ♂.
	0	+ 14	64		2	.....	.....	.....	.....	.....	.....	42
3.....	1	+ 5	.....	45	21	.....	.....	.....	.....	.....	.....	.....
3.....												
	1	+ 19	109		23	.....	.....	.....	.....	.....	.....	42
Total.....												
4.....	10	+ 8	52		40	.....	.....	.....	.....	.....	.....	.....
4.....	1	+ 9	30	47	48	.....	.....	.....	.....	.....	.....	.....
4.....	1	+ 4	6		5	.....	.....	.....	.....	.....	.....	.....
4.....	3	+ 7	42		12	.....	.....	.....	.....	.....	.....	7
4.....	1	.....	26		37	.....	.....	.....	.....	.....	.....	.....
Total....	16	+ 28	203		142	.....	.....	.....	.....	.....	.....	7
5.....	8	+ 9	16		.....	.....	.....	.....	.....	.....	.....	26
5.....	7	+ 1	30		.....	.....	.....	.....	.....	.....	.....	13
5.....	1	+ 4	22		.....	.....	.....	.....	.....	.....	.....	7
5.....	2	+ 1	1		.....	.....	.....	.....	.....	.....	.....	2
5.....	1	+ 4	10		1	.....	.....	.....	.....	.....	.....	8
5.....	9	+ 11	42		22	.....	.....	.....	.....	.....	.....	.....
5.....	11	+ 5	37		21	.....	.....	.....	.....	.....	2	.....
5.....	2	+ 6	2	12	6	.....	.....	.....	.....	.....	2	2
5.....	3	+ 8	3	22	21	.....	.....	.....	.....	.....	.....	1
5.....	7	+ 3	6	9	2	.....	.....	.....	.....	.....	.....	.....
5.....	1	+ 6	14	.....	.....	.....	.....	.....	.....	.....	.....	13
5.....	4	+ 6	31	.....	.....	.....	.....	.....	.....	.....	.....	28
5.....	7	+ 8	28	.....	.....	.....	.....	.....	.....	.....	.....	23
5.....	13	+ 8	28	.....	20	.....	.....	.....	.....	.....	.....	.....
5.....	14	+ 12	37	.....	3	.....	.....	.....	.....	.....	.....	.....
5.....	3	+ 6	75	.....	46	.....	.....	.....	.....	.....	.....	.....
5.....	21	+ 5	.....	29	28	.....	.....	.....	.....	.....	.....	.....
5.....	10	+ 19	.....	42	30	.....	.....	.....	.....	.....	.....	.....
5.....	4	+ 5	.....	16	9	.....	.....	.....	.....	.....	.....	.....
5.....	0	+ 4	7	.....	5	.....	.....	.....	.....	.....	.....	.....
5.....	0	+ 12	36		.....	.....	.....	.....	.....	.....	.....	10
5.....	13	+ 8	28		.....	.....	.....	.....	.....	.....	.....	20
5.....	14	+ 12	37		.....	.....	.....	.....	.....	.....	.....	13
5.....	2	+ 4	16		7	.....	.....	.....	.....	.....	.....	.....
Total....	157	+167	636		221	.....	.....	.....	.....	.....	4	166
6.....	13	+ 13	25	.....	17	.....	.....	.....	.....	.....	.....	10
6.....	9	+ 11	52	.....	44	.....	.....	.....	.....	.....	.....	.....
6.....	10	+ 5	4	35	26	.....	.....	.....	.....	.....	.....	.....
6.....	25	+ 30	187	.....	.....	.....	.....	.....	.....	.....	.....	88
6.....	2	+ 2	10	.....	.....	.....	.....	.....	.....	.....	.....	8
6.....	1	.....	1	7	4	.....	.....	.....	.....	.....	.....	.....
6.....	7	+ 2	11	33	27	.....	.....	.....	.....	.....	.....	.....
6.....	5	+ 1	.....	12	23	.....	.....	.....	.....	.....	.....	.....
6.....	5	+ 7	24	.....	.....	.....	.....	.....	.....	.....	.....	15
6.....	0	+ 8	55	.....	.....	.....	.....	.....	.....	.....	.....	50
6.....	.....	+ 2	12	.....	.....	.....	.....	.....	.....	.....	.....	6
6.....	2	+ 1	28	.....	.....	.....	.....	.....	.....	.....	.....	25
6.....	26	+ 28	108	.....	.....	.....	.....	.....	.....	.....	.....	85
6.....	53	+ 17	144	.....	.....	.....	.....	.....	.....	.....	.....	141
Total....	158	+127	661	87	141	.....	.....	.....	.....	.....	.....	428

TABLE 5.—*X 6 Set*—continued.

Gen.	Both wings Notch ♀.	One wing Notch ♀.	Normal	Eosin ruby ♀.	Eosin ruby ♂.	Eosin Notch ♀.	Ruby ♂.	Eosin ♀.	Notch ruby ♀.	Ruby ♂.	Eosin ♂.	Normal ♂.
7.....	3	+ 1	20	.....	.....	.....	.....	.....	.....	.....	.....	22
7.....	1	+ 2	13	5	.....	.....	.....	.....	.....	.....	.....	9
7.....	34	+ 12	62	.....	.....	.....	.....	.....	.....	.....	.....	51
7.....	16	+ 7	39	.....	.....	.....	.....	.....	.....	.....	.....	45
7.....	16	+ 10	55	.....	.....	.....	.....	.....	.....	.....	.....	27
7.....	2	.....	7	.....	4	.....	.....	.....	.....	.....	.....	1
7.....	17	+ 6	36	.....	.....	.....	.....	.....	.....	.....	.....	29
7.....	17	+ 2	67	.....	.....	.....	.....	.....	.....	.....	.....	46
7.....	45	+ 17	4	82	71	.....	.....	1	.....	.....	.....	1
7.....	5	+ 24	82	.....	.....	.....	.....	.....	.....	.....	.....	38
7.....	7	+ 1	.....	7	5	.....	.....	.....	.....	.....	.....	.....
7.....	4	+ 6	20	16	27	1	.....	1	.....	.....	.....	.....
Total....	167	+ 88	405	110	107	1	.....	2	.....	.....	.....	269
8.....	14	+ 17	4	42	35	.....	.....	.....	.....	.....	2	.....
8.....	3	+ 9	47	.....	3	.....	.....	.....	.....	.....	.....	51
8.....	12	+ 29	98	8	.....	.....	.....	.....	.....	.....	.....	75
8.....	6	+ 16	50	.....	4	.....	.....	.....	.....	.....	.....	37
8.....	20	+ 39	34	72	79	.....	.....	.....	.....	.....	.....	.....
8.....	13	+ 3	5	24	19	.....	.....	.....	.....	.....	2	.....
8.....	15	+ 14	79	.....	1	.....	.....	.....	.....	.....	1	63
8.....	8	+ 24	41	66	78	.....	.....	1	.....	.....	2	.....
Total....	91	+151	358	212	219	.....	.....	1	.....	.....	7	226
9.....	15	+ 4	4	23	26	.....	.....	.....	.....	1	1	.....
9.....	5	+ 5	5	21	17	.....	.....	.....	.....	.....	.....	.....
9.....	13	+ 13	46	.....	.....	.....	.....	.....	.....	.....	.....	40
9.....	4	+ 2	13	37	31	.....	.....	1	.....	.....	1	.....
9.....	3	+ 16	7	44	29	.....	2	2	.....	.....	.....	.....
9.....	17	+ 7	14	36	37	.....	.....	1	.....	.....	3	.....
9.....	14	+ 16	22	53	57	.....	.....	3	.....	1	1	.....
9.....	3	+ 15	16	16	30	.....	.....	.....	.....	1	1	.....
9.....	30	+ 14	10	35	57	.....	.....	.....	.....	2	2	.....
9.....	41	+ 15	23	88	84	.....	4	2	4	2	1	.....
9.....	8	+ 6	8	29	28	.....	.....	.....	.....	.....	.....	.....
9.....	13	+ 16	12	56	50	5	.....	.....	.....	.....	8	.....
9.....	8	+ 6	9	20	14	1	.....	.....	.....	.....	1	.....
9.....	8	+ 12	10	18	13	.....	.....	.....	.....	.....	.....	.....
9.....	4	+ 15	16	45	61	.....	1	.....	.....	1	1	.....
Total....	186	+162	215	521	534	6	7	9	4	8	21	40
10.....	6	+ 15	11	42	45	.....	1	1	.....	.....	.....	.....
10.....	4	+ 20	13	39	1	1	.....	.....	.....	.....	1	.....
Total....	10	35	24	81	46	1	1	1	.....	.....	1	.....
11.....	1	+ 1	2	16	18	.....	.....	.....	.....	.....	.....	.....
11.....	6	+ 13	21	42	33	.....	.....	1	.....	.....	2	.....
11.....	13	+ 23	16	48	46	2	.....	8	.....	.....	3	.....
11.....	2	+ 8	15	18	17	1	.....	3	.....	.....	0	.....
11.....	2	+ 6	14	16	20	.....	.....	.....	.....	.....	1	.....
11.....	9	+ 13	30	70	40	.....	.....	5	.....	1	1	.....
11.....	3	0	22	49	41	.....	.....	.....	.....	1	2	.....
11.....	12	+ 46	84	158	167	1	.....	8	.....	.....	2	.....
11.....	40	+ 22	7	57	52	.....	3	1	.....	.....	4	.....
11.....	13	+ 11	8	32	27	.....	.....	.....	.....	.....	.....	.....
Total....	101	+143	219	406	361	4	3	26	.....	2	15	.....

TABLE 5.—*X 6 Set*—continued.

Gen.	Both wings Notch ♀.	One wing Notch ♀.	Normal	Eosin ruby ♀.	Eosin ruby ♂.	Eosin Notch ♀.	Ruby ♀.	Eosin ♀.	Notch ruby ♀.	Ruby ♂.	Eosin ♂.	Normal ♂.
12.....	13	+ 17	7	55	23	.....	.....	4	.....	.....	.....	.....
12.....	21	+ 79	123	172	178	1+1	.....	18	.....	1	11	.....
12.....	16	+ 25	33	72	70	.....	.....	1	.....	.....	4	.....
12.....	8	+ 12	7	19	24	.....	.....	2	.....	.....	1	.....
Total....	58	+133	170	318	295	1+1	.....	25	.....	1	16	.....
16.....	15	+ 10	17	42	34	.....	1	5	.....	.....	2	.....
16.....	3	+ 1	.....	8	8	1	.....	.....	.....	.....	.....	.....
16.....	7	+ 1	.....	10	12	.....	.....	1	.....	.....	.....	.....
Total....	25	+ 12	17	60	54	1	1	6	.....	.....	2	.....
17.....	.....	+ 5	5	12	13	.....	.....	1	.....	.....	.....	.....
17.....	3	+ 1	1	5	7	.....	.....	.....	.....	.....	.....	.....
17.....	4	+ 11	17	24	23	.....	.....	.....	.....	1	.....	.....
17.....	3	+ 4	1	9	4	.....	.....	.....	.....	.....	.....	.....
17.....	4	+ 7	8	13	13	.....	.....	.....	.....	.....	.....	.....
17.....	6	+ 12	20	21	24	.....	1	1	.....	.....	1	.....
17.....	6	+ 23	21	57	65	.....	.....	1	.....	.....	3	.....
17.....	12	+ 6	24	66	38	.....	.....	.....	.....	1	1	.....
17.....	14	+ 18	12	104	87	+2	.....	1	.....	.....	1	.....
17.....	6	+ 11	11	28	27	.....	.....	2	2	.....	.....	.....
17.....	10	+ 20	17	50	50	1	8	8	.....	10	.....	.....
Total....	68	+118	137	389	351	+3	9	14	2	12	6	.....
18.....	.....	+ 2	8	18	30	.....	.....	.....	.....	.....	.....	.....
18.....	2	+ 4	6	22	20	.....	.....	.....	.....	.....	1	.....
18.....	7	+ 6	16	17	18	1	.....	.....	.....	1	1	.....
18.....	3	+ 13	12	14	10	.....	.....	.....	.....	.....	2	.....
18.....	8	+ 14	9	29	18	.....	.....	.....	.....	.....	1	.....
18.....	1	+ 13	15	27	24	1	.....	2	.....	.....	.....	.....
18.....	14	+ 19	52	69	80	1+3	.....	1	.....	.....	.....	.....
Total....	35	+ 71	118	196	200	1+5	.....	3	.....	1	5	.....
19.....	17	+ 29	56	63	59	.....	.....	16	.....	1	5	.....
19.....	3	+ 3	5	10	3	.....	.....	.....	.....	.....	.....	.....
19.....	17	+ 11	12	37	36	.....	.....	1	.....	1	.....	.....
19.....	10	+ 6	10	36	31	.....	.....	1	.....	.....	1	.....
19.....	.....	+ 9	16	21	13	1	.....	.....	.....	.....	.....	.....
19.....	2	+ 1	18	14	18	1	.....	1	.....	.....	3	.....
19.....	11	+ 6	10	35	22	.....	.....	3	.....	.....	.....	.....
19.....	7	+ 8	12	19	22	.....	.....	.....	.....	.....	.....	.....
19.....	3	+ 5	4	17	8	.....	.....	.....	.....	.....	.....	.....
19.....	.....	+ 78	143	252	212	2	.....	22	.....	2	9	.....
Total....	70	+156	286	504	424	4	.....	44	.....	4	18	.....

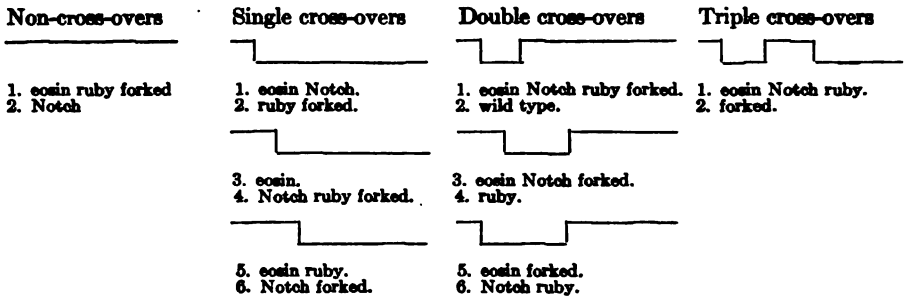
wing were chosen as parents. Progress was slow, but there are clear indications at the end that the stock was different. Little progress was made until, during the eighth generation, the phenotypic normal females were used as parents. From the ninth to twelfth generation inclusive a marked addition to the phenotypic normal class became evident in most of the cultures. At the end of the selection, males of this stock were bred to females of the other selected line (SS, 162).

Had different modifying factors been present, the atavistic type of Notch should have been shown by the daughters, but if both lines had been changed through the isolation of the same modifying gene, the results are expected to be the same as when the male comes from the same line as the Notch female. The cross showed that the Notch modifier was the same in both lines.

LOCALIZATION OF THE GENE FOR NOTCH.

Earlier evidence had shown that the gene for Notch lies in the X-chromosome somewhere in the region between eosin and ruby. The following "three point" experiment was devised to furnish more precise data. The red-eyed Notch female was bred to an eosin ruby forked male. Her Notch daughters are expected to contain one X chromosome with the Notch locus and the other X chromosome to contain the eosin ruby forked loci. The approximate location of these loci is that shown in figure 92.

The figure also indicates that three possible regions of crossing over occur between the three pairs of genes involved. There are sixteen possible classes: two non-cross-overs, six single cross-overs, six double cross-overs, and two triple cross-overs. The characters shown by each of these classes are the following:



When an F_1 Notch of this composition is crossed to an eosin ruby forked male (the multiple recessive) all the classes of gametes produced by such a female will be revealed both in the female and in the male offspring, except that there will be only half as many classes of males as of females, since all those males that get the gene for Notch will die. In the table, the male classes are entered separately from the female classes. It was anticipated that calculations based on the males alone would be more accurate than those based on the females alone, because in the latter sex there is some difficulty in separating the eosin from the ruby females, while in the males no such confusion is possible. The computations show, however, that the differences between the two sets of data are as near as is to be expected for the numbers involved. Therefore the estimates based on the total figures are probably to be preferred.

There is a small chance of contamination from the food when so many cultures as these are carried out, even although all the ordinary precautions are taken. Thus the four normal males that appeared are under suspicion. Two of these were tested to the second generation and found to contain no other than normal genes. Since the male contains only one sex-chromosome, it was to be anticipated that such red-eyed normal males would not contain any other sex-linked genes than they show unless something unusual had occurred. It is, however, conceivable that a lethal-bearing male rarely comes through (as happens in the case of a few other lethals) even although no notch is observable in the wing. Were this possible some of his daughters or granddaughters would be expected to show Notch, but as none did so, the presumption is that these red-eyed males were not of this kind. It is also possible to mistake at times an old ruby-eyed fly for a red-eyed fly if only a casual examination is made, but as it was appreciated that no red-eyed male was expected, a careful scrutiny of the red males was made. For these and other reasons I have discarded the two untested males of the four from the general calculation in locating the factors, although I have also given the calculations in which these are included. The differences in the two results are too small to be of significance.

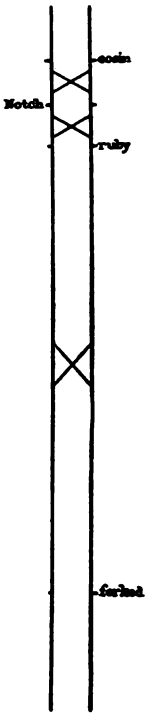


FIG. 92.

A similar doubt arises about the corresponding double cross-over classes in the females that gave two eosin Notch ruby forked females and two normal females. Both of the latter were tested with eosin ruby forked males and gave normal sex-ratios and no Notch daughters. All the daughters and sons had red eyes. For these three reasons there can be little doubt that both of these females in question were due to contamination by wild-type flies.

The other two daughters can not be so easily dsmissed, because they were obviously not due to contamination, since they showed all of the genes involved in the experiment. Unfortunately I have no records to show whether they were tested, or, if so, whether they lived. It is true that occasionally flies are found that have a nick in their wings due to accident or to some other mutation, and in numbers as large as those here employed, the occurrence of such flies is to be expected. It is to be regetted that I was not aware of the fact that Notch flies (even those phenotypically normal for wing margin) can be identified, under the microscope, by the thicker second and fifth veins. By this means the two normal eosin notch ruby forked females could have been securely identified. While it is highly probable that the same difference holds for the selected notch, this has not been deter-

TABLE 6.

	Females.																Males.							
	Not eosin.								Eosin.								Not eosin.				Eosin.			
	Not Notch.				Notch.				Not Notch				Notch.				Not Notch.				Not Notch.			
	Not ruby.		Ruby.		Not ruby.		Ruby.		Not ruby.		Ruby.		Not ruby.		Ruby.		Not ruby.		Ruby.		Not ruby.		Ruby.	
	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.	Not f.	f.
1	....	..	....	1	41	29	0	1	4	0	27	37	....	..	....	..	....	..	....	..	2	2	39	31
2	....	..	....	1	44	30	1	0	2	1	32	63	....	..	....	..	1	1	0	1	....	..	35	50
3	1	0	0	1	50	37	1	0	2	1	28	41	0	1	....	..	1	0	....	..	3	1	19	49
4	....	..	....	1	20	14	....	....	1	0	19	20	....	..	....	..	....	..	....	..	....	..	13	21
5	....	..	....	....	30	24	0	4	1	0	41	37	2	0	....	..	....	..	0	1	2	2	26	42
6	....	..	....	....	8	4	1	0	....	....	7	7	....	..	....	..	....	..	1	0	....	..	3	8
7	....	..	....	....	36	21	....	....	1	3	34	25	....	..	....	..	....	..	....	..	1	1	26	30
8	....	..	0	1	37	29	....	....	....	....	26	42	1	0	0	1	....	..	....	..	3	0	24	22
9	....	..	....	....	21	17	0	2	0	1	20	17	0	1	....	..	....	..	0	1	....	..	19	30
10	....	..	....	....	13	9	0	1	2	0	6	15	....	..	....	..	....	..	....	..	....	..	6	21
11	....	..	....	....	24	16	0	1	1	0	19	14	....	..	....	..	....	..	....	..	....	..	19	19
12	....	..	1	0	14	6	....	....	....	....	11	7	....	..	....	..	....	..	0	3	2	0	9	10
13	....	..	....	....	18	11	....	....	2	1	6	11	....	..	....	..	1	0	....	..	....	..	6	10
14	1	0	....	....	5	8	1	0	....	....	2	5	....	..	....	..	1	0	....	..	....	..	2	4
15	....	..	....	....	23	19	0	0	0	0	20	26	....	..	....	..	....	..	....	..	1	0	16	24
16	....	..	....	1	4	3	0	0	2	0	4	5	....	..	....	..	....	..	....	..	....	..	10	10
17	....	..	....	2	10	13	0	0	0	0	17	22	....	..	....	..	....	..	0	1	1	1	3	11
18	....	..	0	2	16	18	0	1	2	0	16	15	....	..	....	..	....	..	0	1	3	2	21	21
19	....	..	0	1	51	17	....	....	1	1	39	47	1	0	0	1	....	..	....	..	3	0	31	47
20	....	..	0	1	6	5	1	1	1	0	8	9	....	..	....	..	....	..	0	1	0	1	4	17
21	....	..	0	0	27	32	1	1	4	0	23	28	....	..	....	..	....	..	....	..	4	1	16	37
22	....	..	....	....	20	11	0	2	5	2	12	13	1	0	....	..	....	..	....	..	....	..	11	16
23	....	..	....	0	4	7	0	0	....	....	5	21	0	2	....	..	....	..	....	..	....	..	7	26
24	....	..	1	0	12	15	0	1	1	0	14	32	....	..	....	..	....	..	....	..	3	1	12	21
25	....	..	0	1	8	2	....	....	1	0	13	10	....	..	....	..	....	..	....	..	1	0	10	10
26	....	..	....	....	5	7	....	....	2	0	8	7	1	0	....	..	....	..	....	..	1	1	3	5
27	....	..	....	....	19	15	....	....	0	1	13	16	0	1	....	..	....	..	....	..	3	1	19	31
28	....	..	....	....	5	4	....	....	1	1	6	6	....	..	....	..	....	..	....	..	....	..	4	1
	2	0	2	13	571	423	6	15	36	12	476	598	6	5	....	2	4	1	1	9	33	14	413	624

mined, because the original stock of Notch was allowed to die out, and the new Notches that have since appeared are not certainly the same Notch. The new Notches so far as tested, appear to be deficiencies. (See Mohr, O. L., 1919, Genetics, in press.)

There were two classes in the males where crossing-over occurred between eosin and Notch containing 9+14=23 cross-overs (omitting 2+2=4 double cross-overs discussed above). Dividing these 23 by the total number of males, viz, 1,095, gives 2.1 per cent of crossing-over. If the four questionable individuals are utilized the result is 2.4 per cent.

There were two classes in the males where crossing-over took place between Notch and ruby, containing 33+1=34 cross-overs (omitting the four individuals as before). Dividing, then, 34 by the total number

of males, 1,095, gives 3.1 per cent of crossing-over. (If the four questionable individuals are utilized the result is 3.4 per cent.)

There were three classes in the males where crossing-over occurred between Notch and forked, containing 413+14+1=428 cross-overs (omitting the questionable classes). Dividing these by the total number of males, 1,095, gives 39.1 per cent of crossing-over.

In the females there were two classes where crossing-over occurred between eosin and Notch, 19+7=26 (omitting the rejected classes). Dividing 26 by the total number of females, viz, 2,125, gives 1.2 per cent of crossing-over.

In the females there were two classes where crossing-over occurred between Notch and ruby, containing 51+18=69 cross-overs (omitting the rejected females). Dividing these 69 by the total number of females, viz, 2,125, gives 3.25 per cent of crossing-over.

In the females there were three classes where crossing-over occurred between Notch and forked, viz, 859+18+7=884. Dividing these by the total number of females, viz, 2,125, gives 41.6 per cent of crossing-over.

THE IDENTIFICATION OF THE MODIFYING GENES.

The following method, which has come into use in this laboratory as the best and quickest method to identify modifying genes in the second or third chromosome, takes advantage of two dominant genes, one in each of these chromosomes, as well as of the fact that there is no crossing-over between the members of any pair of chromosomes in the male.

The three chromosomes of the Notch female that are involved are represented in the left top line in figure 93. The gene for Notch is in one X chromosome and the genes for eosin and ruby in the other X. The second and the third chromosomes are supposed to carry the modifying gene or genes, whose presence there this experiment is designed to test.

The chromosomes for the Star Dichæte (S′ D′) male are shown in the second line. The X chromosome carries only normal genes, while the second chromosome carries the gene for Star (S′) in one member of the pair and its normal allelomorph in the other member; the third chromosome carries the gene for Dichæte in one member of the pair and its normal allelomorph in the other. Neither Star nor Dichæte are viable in homozygous condition; hence, as stated, one member of each of the pairs of chromosomes that carry these dominant genes is Star or Dichæte respectively, the other normal.

Therefore, when such a male is crossed to the selected Notch female, all the Star Dichæte sons have received the Star and Dichæte genes (and their respective chromosomes) from the father and the homologous chromosomes from the mother. The single X chromosome that the

male gets is the eosin-ruby-bearing X chromosome of the mother (the other males die). In other words, their composition is that represented in the third line to the left in figure 93.

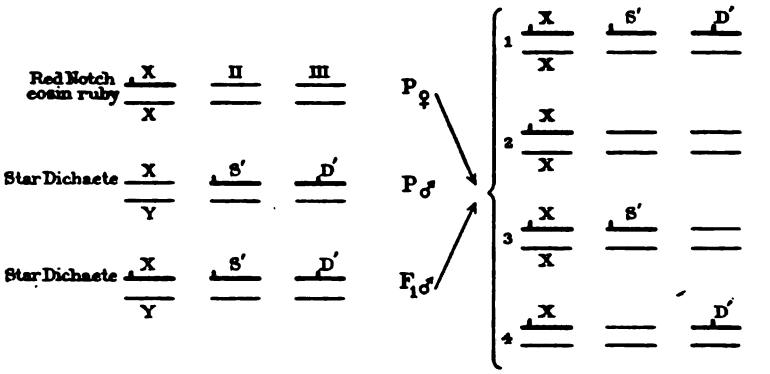


FIG. 93.

If this male is now back-crossed to a selected Notch female (see figure 93) any red-eyed Notch daughter that is also Star-Dichæte (upper line to right; No. 1) must have gotten the Star (II) and the Dichæte (III) chromosomes from her father (neither of which bears the modifiers sought for) and an X chromosome also from the father with genes for eosin ruby eyes and normal wings. She must also have gotten the second and third chromosomes that may carry in one or in both the modifiers sought for (which are recessive) from her mother, as well as an X chromosome bearing the genes for red-eye and Notch wing. Hence such a female should be atavistic Notch, because either the S′ or D′ genes will bring in the normal allelomorph of the postulated modifiers in II and III. Conversely, females that are not Star and not Dichæte (No. 2) should be of the selected type, since their second and third chromosomes, one or both, contain the modifiers.

Continuing the analysis, it is evident that if the modifier (one or more) is in the second chromosome, then all Star Notch daughters (No. 3) should be atavistic, and all not-Stars (No. 4) the selected type of Notch; and if the modifier is in the third chromosome, then all Dichæte Notch daughters should be atavistic (No. 4) and all not-Dichæte (No. 3) selected type of Notch.

The ability to pick out atavistic flies from selected-type flies is essential to this test. In general, this can be done successfully, with, however, a margin of error, but the error is expected from the information at hand to be so small as not to effect the main result. Moreover, the occurrence of red-eyed females with normal wings (flies that are known from the linkage relations of the experiment to have the Notch gene) in any of the classes named above is an almost certain index of the occurrance of the modifier.

The results of such a test are given in table 7. The table includes only females and only the red-eyed females (the flies that are *genetically* Notch), while the eosin ruby females and all of the males were thrown away. Examination of the table shows that practically all of the not-Star, not-Dichæte females have normal wings (potentially

TABLE 7.

	Not star ♀.						Star ♀.					
	Not-Dichæte.			Dichæte.			Not-Dichæte.			Dichæte.		
	Notch Sel'ed.	Norm. Sel'ed.	Atavistic.	Notch Sel'ed.	Norm. Sel'ed.	Atavistic.	Notch Sel'ed.	Norm. Sel'ed.	Atavistic.	Notch Sel'ed.	Norm. Sel'ed.	Atavistic.
A...	2+1	6	.....	.....	.....	9	+2	1	.....	.....	.....	7
A...	1+2	5	.....	.....	.....	1	+2	7	.....	.....	.....	6
A^a..	1+4	7	.....	+1	2	8	+1	8	.....	+1	.....	13
A^b..	1+1	6	.....	.....	.....	10	.....	6	.....	.....	.....	12
A^3...	+2	3	.....	.....	.....	5	+1	3	.....	.....	.....	3
A^3...	3+1	5	.....	.....	.....	5	+1	7	.....	.....	.....	10
A^p..	1+1	.....	.....	.....	.....	4	+1	3	.....	.....	.....	2
A^s..	2+4	22	.....	.....	.....	18	+1	27	.....	+3	.....	18
A^o..	5+7	2	.....	.....	.....	7	2+2	5	.....	1	.....	9
B...	.....	3	.....	.....	.....	1	+1	1	.....	.....	.....	6
C...	2+6	4	.....	.....	.....	13	+5	6	.....	2	.....	16
D...	.....	.....	.....	.....	.....	.....	1	2	.....	.....	.....	3
E...	1+6	4	.....	.....	.....	9	+2	9	.....	+2	.....	14
F...	+4	5	.....	.....	.....	6	+2	4	.....	1	.....	4
H...	3+7	11	.....	.....	.....	27	2+2	8	.....	.....	.....	27
K...	4+8	19	.....	.....	.....	18	1+5	17	.....	.....	.....	18
P...	+3	3	1	.....	.....	.....	+1	6	.....	.....	.....	5
P^s...	+5	7	.....	.....	.....	11	.....	2	.....	.....	.....	3
	26+62	112	1	+1	2	152	6+29	122	.....	4+6	.....	176

Notch).[1] This is the class that contains the original second and third chromosomes and their modifying genes if such were present. Conversely, practically all of the Star-Dichæte females are atavistic, and this class contains the Notch females that have received the second and the third chromosomes from the Star-Dichæte males. Thus far the evidence shows that the change that took place during selection is caused by something in one or the other or both of these two chromosomes. Whether both or only one is shown by further analysis of the results. For instance, the fact that all the Dichæte flies are atavistic, and the fact that all not Dichæte are selected type, shows that the modifier is in the third chromosome. Had the modifying gene or genes been in the second chromosome, then all Star-eyed females should be atavistic, which they are not, and, conversely, all not-Star-eyed females should be selected type, which they are not. Hence the modifier in question is not in the second chromosome.

Finally, the same evidence proves that the modifiers that caused the change are not in the sex chromosome as recessive modifiers be-

[1] In this table (also in tables 4 and 5) the + sign indicates that the number of flies that follow were notched in only one wing.

cause the not-Star not-Dichæte females are practically all the selected type, and the Star-Dichæte are practically all classified as atavistic; yet the females of both classes contain the same Notch-bearing chromosome that must be identical, since in both it is the X chromosome of the selected stock.

In the F_1 generation (table 8), the parents of the flies in table 7, it was found that all of the Notch females were atavistic as expected. In some sets the extent to which notching was developed was greater than in others. It is important for present purposes to note that there is no difference in the extent of development of the character Notch in the Dichæte and in the not-Dichæte (straight-winged) females. This means that the wing-character Dichæte does not modify the Notch character when present with it. Consequently we should not expect in F_1 any difference between Dichæte and not-Dichæte Notch females, due to the Dichæte gene.

TABLE 8.

	Not-Dichæte ♀.		Dichæte ♀.		Not-Dichæte ♂.			Dichæte ♂.
	Notch ♀.	Not-Notch ♀.	Notch ♀.	Not-Notch ♀.	Eosin ruby ♂.	Eosin ♂.	Ruby ♂.	
A–16241..	18 atavistic.....	17	7+1 atavistic.....	13	9	.....	.....	9
B–1624...	8 not very atav.....	14	4+3 not very atav..	12	12	1	.....	13
C–16241..	6+1 atavistic.....	16	3 atavistic.....	15	19	.....	.....	11
E–1624...	12 not very atav.....	18	3+1 atavistic.....	8	16	.....	.....	2
F–1624...	13 atavistic.....	26	15+3.....	21	22	.....	1	6
G–16241..	15+2 not very atav..	22	3+4.....	25	16	1	1	0
K–1624...	7 atavistic.....	0	3+1.....	6	12	.....	.....	10
M–1624...	4 atavistic	4	2+1 not very atav..	6	6	1	.....	2
Total..	83+3.....	117	40+14.....	106	112	3	2	53

SHORT NOTCH.

Several times in the early history of the Notch stock, females appeared with wings much shorter than those of the atavistic type that can be obtained at any time by out-crossing selected Notch. The general character of the short-Notch wing may be gathered from figure 94, *a*. Not only is the wing shorter and broader, but the end is more abruptly and fully squared off than in the typical stock or in the atavistic Notch, figure 94, *b*.

Many of the females are sterile, so that the stock has nearly died out several times when pairs were used, but mass-cultures of this type can be kept going. Several times, when even shorter winged individuals have appeared, and I have attempted to breed from them, I have found that they were sterile. The stock was first red-eyed, but later eosin eye was introduced, so that the X chromosome bearing

Notch has as its mate in the female an X chromosome bearing eosin. Such a female crossed to an eosin brother gives 1 red-eyed Notch ♀ ; 1

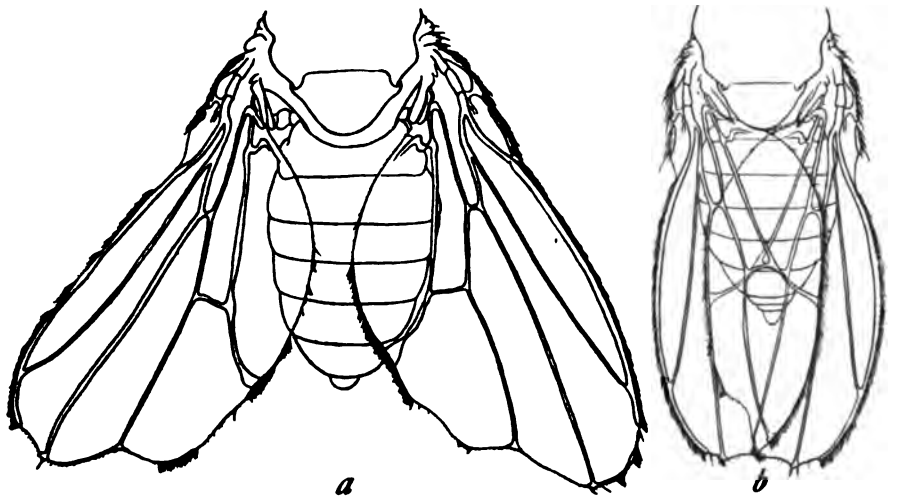


FIG. 94.

eosin ♀ : 1 eosin ♂, and the expected number of cross-overs. The stock was kept running by breeding in every generation a number of short-Notch females to some of their eosin brothers, the eosin sisters being rejected. No special effort was made to pick out the shortest of the Notch females. The general run of the stock may be gathered from table 9 for the fourth, fifth, and sixth generations. For some time

TABLE 9.—*Short Notch.*

	Notch ♀.	Eosin ♀.	Eosin ♂.	Wild-type ♂.		Notch ♀.	Eosin ♀.	Eosin ♂.	Wild-type ♂.
F_4...	30	48	46	.....	F_5...	30	41	29	.....
F_4...	20	10	18	5	F_5...	47	57	41	.....
F_5...	24	13	13	.....	F_5...	39	36	58	.....
F_5...	29	41	36	.....	F_5...	43	32	30	1
F_5...	23	22	30	.....	F_5...	52	62	61	.....
F_5...	21	26	22	1	F_6...	54	54	59	1
F_5...	84	71	78	.....	F_6...	29	27	31	.....
F_5...	4	27	24	1	F_6...	18	24	36	1
F_5...	35	25	28	1	F_6...	63	53	76	.....
F_5...	30	53	34	.....	F_6...	86	55	60	.....

I thought that short Notch might be an allelomorph of Notch—a difficult point to settle if it were, because there are no males of either class to bring the two allelomorphs together, and no other way of getting them into the same individual. But if the shortness of this type is due to a modifying gene it should be carried by the not-Notch males as well as by the female, and its presence could be demonstrated by crossing.

When a short Notch female is out-crossed to a wild male, the daughters are *atavistic* (fig. 94, *b*), which proves that short Notch is not due directly to a dominant Notch unless the wild male brings in a dominant gene modifying such a dominant gene. If it does, then the next F_2 generation should give 3 short to 1 atavistic. On the other hand, if short Notch is due to a recessive modifier, the F_2 ratio should be the reverse, namely, 3 atavistic to 1 short. It may be stated here that the evidence shows that a recessive modifier is present, but present in the sex-chromosome itself, so that the numerical results follow the expectation for sex-linked inheritance. The following tests were made to discover the location of the modifying factor for short Notch:

FIRST TEST.

(1) A short Notch female was crossed to a Star Dichæte male. The Star Dichæte sons of this cross get their X chromosome from their mother, as well as one normal autosome carrying the normal allelomorph of Star and another that of Dichæte. The fourth chromosome pair may be left out of account. When such a son is back-crossed to a short Notch stock female, every Notch daughter will have one X from her mother and one from her father (which in turn came from his mother, hence from the short Notch stock). In other words, all Notch daughters have the same X chromosomes as the short Notch stock females. But some of the Notch daughters will have one Star-bearing second chromosome and one normal second chromosome; others both normal of stock. If a recessive factor for short Notch was in the second chromosome, the latter, containing both such chromosomes, should give a shorter wing than the former. Similarly for Dichæte. Some of the Notch daughters will have a Dichæte and a normal third chromosome, others both normal chromosomes of the short stock. If the modifier (shortener) is in the third chromosome the latter (both chromosomes present) should be shorter than the former, etc. The results are given in table 10.

This table shows (1) that the short Notch reappears in this second generation (back-cross); (2) that it is not more common in the not-Star than in the Star, which means that the modifier is not present in the second chromosome; (3) that it is not more common in the not-Dichæte than in the Dichæte, which means that the modifier is not in the third chromosome; (4) it follows that it must be present either in the first or the fourth. The second test (below) will show that there is in fact an important modifier in the X chromosome itself. Whether another is present in the fourth chromosome will be examined later when the atavistic Notch flies that also occur in table 10 will be discussed.

It will be noticed in table 10 that in addition to the short Notch there are others called intermediate and even atavistic. That these are for the most part due to fluctuations of the short-Notch character itself is almost certain, since even in stocks bred for 20 generations for short Notch a similar range occurs in some bottles, but when tested the "atavistic" (or more generally the "intermediate" Notch) give the same kinds of daughters as do their sisters, the short Notch females.

TABLE 10.

Bot. No.	Notch females.				Not-Notch females.								Males.
	Not-Star.		Star.		Not-Star.				Star.				
	Not-Dich.	Dich.	Not-Dich.	Dich.	Not-Dich.		Dich.		Not-Dich.		Dich.		
	Not w^e.	Not w^e.	Not w^e.	Not w^e	Not w^e.	w^e.	Not w^e.	w^e.	Not w^e.	w^e.	Not w^e.	w^e.	
CE.	7 short....	13 short.... 2 atavistic.	9 short..... 6 intermed.. 2 atavistic..	10 short.... 6 atavistic.	1	24	....	20	....	1	1	8	49
CA.	1 short....	4 short....	.....	1 short....	....	5	....	3	....	....	....	4	4
CA.	.....	2 short....	3 short.....	1 (?).....	....	6	....	....	....	....	1	....	6
CX.	3 short.... 2 atavistic.	1 short....	4 atavistic..	3 atavistic.	1	14	....	12	....	....	....	3	29
CX.	.....	5 atav. or short.	.....	2 atav. or short.	....	3	....	6	....	....	....	15	4

SECOND TEST.

In this test the reciprocal cross was first made, viz, Star Dichæte female by eosin male of short-Notch stock. The F_1 male gets his X chromosome from his Star Dichæte mother. Consequently if it is the X chromosome in the short-Notch stock that carries a recessive modifier for Notch (that has both the Notch-bearing X and its normal homologous chromosome) no short-Notch daughters are expected when the above F_1 male is mated to a short-Notch stock female.

The result of mating such F_1 Star Dichæte males to short-Notch females is shown in table 11. Practically all of the Notch females are atavistic or slight Notch. The result is in striking contrast to the result in the first test and means, obviously, that the X chromosome contains one or more modifiers that shortens the effects of the Notch factor itself. That some of the Notch females are atavistic and some slight may be expected, since separation of the two classes is difficult or impossible and no emphasis can be laid on the classification as it stands.

THIRD TEST.

In the second generation of the first test there were some eosin Star Dichæte males whose X chromosome should be the same as that of the F_1 males. Since the second and third chromosomes appeared not to affect the result in the first test it should not be expected to affect the result here, whichever ones are present. Half of the sperm of the males, however, should carry one of the fourth chromosomes of the short-Notch mother; the other fourth chromosome would be in half of the flies derived from the same source and in half from the Star-

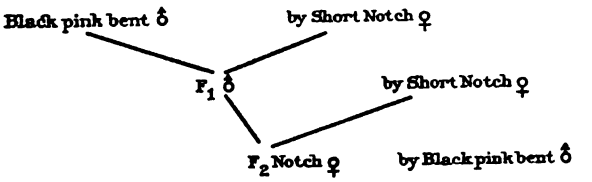
TABLE 11.

Bot. No.	Not-Notch females.								Notch females.				Males.
	Not-Star.				Star.				Not-Star.		Star.		
	Not-Dich.		Dich.		Not-Dich.		Dich.		Not-Dich.	Dich.	Not-Dich.	Dich.	
	Not E.	Eosin.	Not E.	Eosin.	Not E.	Eosin.	Not E.	Eosin.	Not Eosin.	Not Eosin.	Not Eosin.	Not Eosin.	
OA.	29	3	24	3	7	....	7	....	.....	.....	4 (?).....	4 atavistic.. 2 slight.	48
CQ.	14	....	11	....	22	....	7	....	4 atavistic. +1 slight..	5 atavistic.. 4+1 slight. 1 short.	6+3 slight.. or atavistic	.....	46
ZZ..	20	....	25	....	10	....	....	....	5 atavistic. 7 slight....	4 atavistic.. 9 slight.....	7 atavistic..	3 atavistic.. 6 slight.	91
GP.	11	4	8	3	....	....	....	2	.....	1 intermed.. or short.	.....	.....	17
HK.	24	....	44	....	23	....	27	....	5 atavistic.	2 short.....	1 atavistic..	3 atavistic.. 1 short or atavistic.	125
BO.	6	....	8	....	7	....	6	....	3 atavistic.	1 atavistic.. 2 slight.	5 atavistic..	6 atavistic..	33
BO.	....	....	1	....	....	....	....	....	1 atavistic.	.....	.....	3 atavistic.. 1 short.	.....

Dichæte grandparent. If the fourth chromosome causes the difference between the short-Notch and the atavistic type it would be expected that some of the males crossed to short-Notch females would give one result and some another (3 : 1). There were five cultures with both atavistic and short and one with short Notch only. It is doubtful if this should have any significance (although it tallies with the expectation) because it is not absolutely certain that in all of them only one male fertilized the female. The next test was decisive, so that it was not necessary to repeat the experiment.

FOURTH TEST (FOR FOURTH-CHROMOSOME MODIFIERS).

The following method of finding out whether a modifying gene is present in a particular chromosome was suggested by Dr. A. H. Sturtevant. A short-Notch female is first crossed to a black pink bent male. The F_1 males[1] are then crossed (in pairs) to stock short-Notch females (see scheme below).



Since the F_1 male had one fourth chromosome from black pink bent (and since there is no crossing -over in the male), half of his Notch (F_2) daughters will have this chromosome (only one each), and half will not have it. If they are of two sorts (so classified in table 12), such as intermediate and short Notch, their differences might depend on the presence of the bent fourth chromosome in half of the Notch females. If now we separate as far as possible the females into two classes, and

TABLE 12.—*F_1 ♂ (out of short Notch ♀ by bent ♂) by short Notch ♀.*

	Normal ♀.	Short Notch ♀.	Interm. Notch ♀.	Eosin ♀.	Eosin ♂.	Eosin-Notch ♀.	Normal ♂.
1....	2	15	68	73	85	.....	.....
2....	.....	66	41	122	102	1 (interm.)	2
3....	.....	21	28	47	30	.....	.....
4....	.....	22	30	60	64	.....	.....
		12	28	32	30	.....	.....
5....	.....	4	30 (2 almost atavistic).	40	27	.....	.....
6....	.....	13	6	14	33	1 (short)	
7....	.....	12	36	51	52	.....	.....
8....	.....	27	15	29	41	.....	.....
				24	.....	.....	.....

test each female separately to find out if she has or has not a bent fourth chromosome, we should get an answer to our question. Each female was bred to a black pink bent stock male. The presence of black, of pink, and of bent (separate or combined) in the offspring was recorded. Table 13 gives the end-results; the first column records the kind of F_2 female tested, the next three columns the kind of notch, and the next three columns indicate (by ×) whether black, pink, or bent was present; and the last column the total number of flies.

[1] Their sisters that were not recorded here were all atavistic Notch.

Inspection of table 13 shows no significant correlation between the kind of Notch shown by the F_2 mother and the presence in it of the bent chromosome derived from the black pink bent stock; for six F_2 short Notch females had this bent chromosome, four did not; six F_2 intermediates did not have it and two F_2 did have it. There is no correlation with black or pink either, hence there were no dominant autosomnal modifiers in black pink bent stock.

TABLE 13.—F_2 *Notch* ♀ *by black pink bent* ♂.

No.	Kind of F_2 ♀.	Notch.			Bent.	Pink	Black.	Total ♂ ♀.
		Short.	Intermediate.	Atavistic.				
1.....	Short.....	6	4	.....	×	×	0	33
2.....	Do	5	2	.....	0	×	0	20
3.....	Do	2	1	.....	×	0	×	33
4.....	Do	3	1	.....	0	0	×	12
5.....	Do	15	0	.....	×	0	0	76
6.....	Do	3	4	.....	×	0	×	15
7.....	Do	14	0	.....	×	×	0	47
8.....	Do	10	1	.....	×	×	×	20
9.....	Do	5	1	.....	0	0	×	17
10.....	Do	12	1	.....	0	0	×	50
11.....	Do	9	5	.....	0	×	×	33
12.....	Intermediate..	2	3	.....	0	0	×	3
13.....	Do	4	19	.....	×	0	×	75
14.....	Do	11	2	.....	0	0	×	49
15.....	Do	11	6	.....	0	0	×	53
16.....	Do	18	2	.....	0	×	×	40
17.....	Do	7	6	.....	×	0	×	59
18.....	Atavistic	23	13	.....	0	×	×	97
19.....	Do.(almost).	2	10	.....	0	×	0	21

RECOMBINATION OF BENT AND SHORT NOTCH.

As a matter of curiosity, largely, the possible interaction of "bent" in double dose on Notch was determined. Flies of the desired recombination were made by crossing bent males to short-Notch females and then by breeding the F_1 Notch females to their brothers. Amongst the F_2 flies were pure bent females that were also Notch. These showed the widest possible range of modification as does the bent character itself. In extreme cases, as shown in figure 95, the wing is as narrow as stumpy wings (see Critique of Theory of Evolution, p. 11, figure 5, *c*, *d*). It is noticeable, too, when looking through such a series, that the extent to which the bent factor manifests itself in other ways, such as in the shortening of the legs, is a sort of index of the extent to which the wing is affected. This might, on first thought, be interpreted as furnishing evidence in favor of the view that the extent to which a character develops may be an index of the quantity of the gene present

in the egg. But since bent flies with the character well developed may not produce, when bred to each other, any more flies of their own kind than do their normal appearing brothers and sisters, there is nothing gained by making such an assumption. On the contrary, it seems more reasonable, I think, to suppose that the same environment (in the widest sense) that is favorable to the full development of the bent characters make that character the more effective in its influence on short Notch. It seems to me hazardous to base any view concerning the nature of the gene itself on evidence of this kind, as has been done by several recent writers.

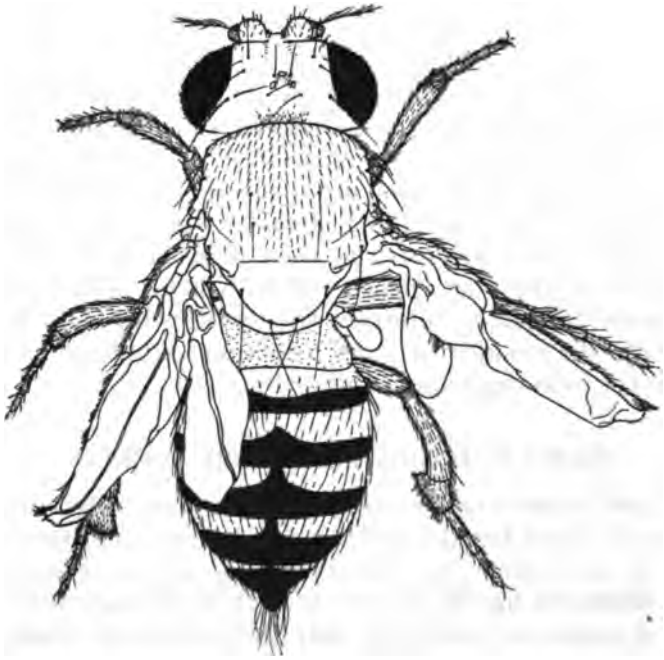


FIG. 95.

CROSSES BETWEEN SHORT NOTCH AND OTHER STOCKS.

Unless the modifying factor for short-Notch is partially dominant or unless other stocks carry the modifier, or some other dominant modifier, the expectation on crossing short-Notch females to males of other stocks is atavistic types of Notch females. The males of short-Notch stock carry in their sex chromosome, as has been shown, a modifier for short-Notch; hence crossing of such males to selected or to atavistic Notch are expected to show some influence on the character of Notch in their daughters if the factor is dominant or partly so. In the light of these expectations the following crosses are not without interest.

SHORT NOTCH BY STAR DICHÆTE.

There were four crosses of this combination that gave in F_1 Notch females with intermediate wings shorter, on the average, than the atavistic type, and therefore more on the order of the short type. The F_1 records are as follows:

Notch-Dichæte ♀.	Notch ♀.	Normal ♀.	Normal ♂.	Dichæte ♂.	Dichæte ♀.
11	17	11	8	11	9
.....	6	8	9	9	5
8	24	17	14	4	7
15	2	4	3	.....	.....

In these results the condition of the wings was the same in the Dichæte female and in those not-Dichæte, showing that the gene of the latter does not itself act as a modifier. This information is of value in working out the location of the modifier by means of the S′ D′ test. The further fact that the F_1 females were "intermediate" between atavistic and short-Notch is, then, more probably due to some other modifier of the Star Dichæte short Notch. This complication makes it more difficult to interpret the results when Star Dichæte is used to locate the modifier of short Notch, but still leaves such a test possible, as the following experiments show.

SHORT NOTCH BY EOSIN RUBY FORKED.

When short-Notch females were crossed to eosin ruby forked males of stock the F_1 Notch females had in nearly all cases shorter wings than the atavistic notch flies (or ordinary Notch) obtained in other cases (four are drawn in fig. 96, *a*, *b*, *c*, *d*). In these instances the eosin ruby forked males may carry not only the normal allelomorph of the selected modifier, but also another gene that carries the Notch towards the short-Notch direction, which might be the modifier in the X chromosome of the father. The F_2 generation came from the short-like Notch F_1 female by her brother. The records are as follows:

	Notch.	Normal.
1.....	33 intermediate short...	56
2.....	90 atavistic	108
3.....	91 short.....	76

The failure to sharply distinguish between the types of Notch in these F_2 counts shows that without first purifying the eosin ruby

forked stock, that stock is not suited to test the location of the regular modifier for short Notch. The experiment was not carried further.

Reciprocally, when the male of short-Notch stock was crossed to selected female, the F_1 Notch females were atavistic, indicating that the Notch gene of the selected stock has not itself changed, and that

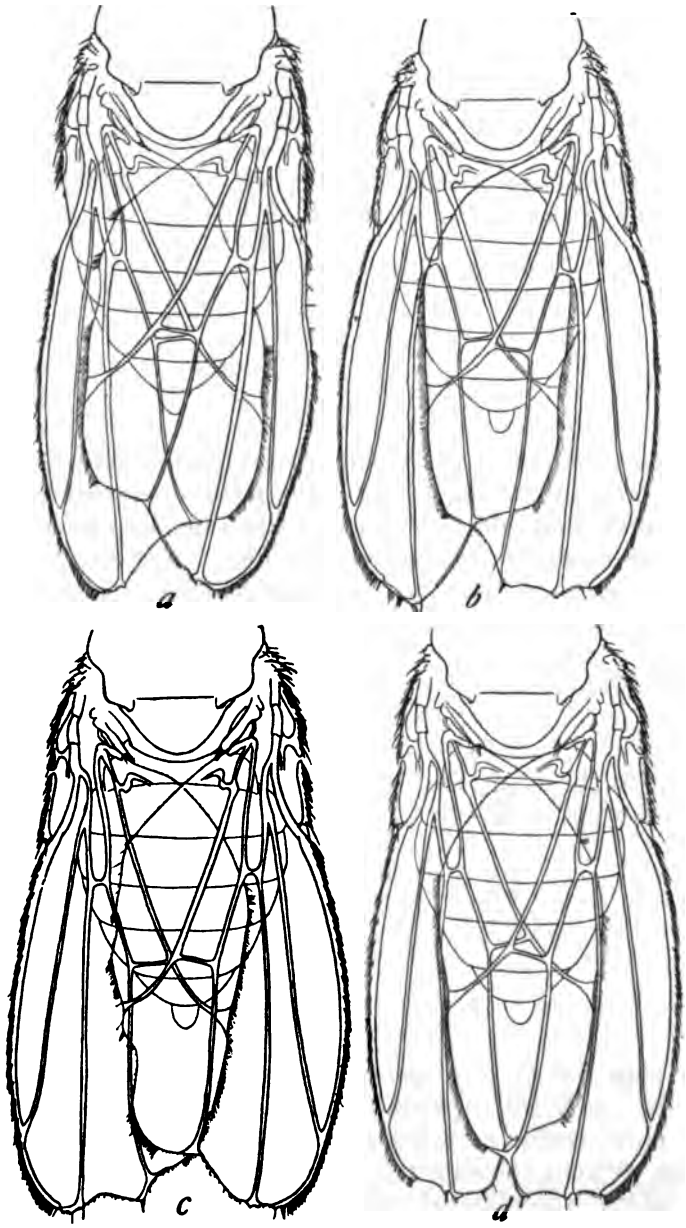


FIG. 96.

the short type carries the normal allelomorph of the selected modifier. (Fig. 97, *a*, *b*, *c*, *d*.) The F_1 counts gave:

Notch (atavistic).	Normal ♀.	Normal ♂.
17.....	18	9
19.....	17	20
8.....	13	5
24.....	33	38
15.....	14	11
16.....	22	7

The reciprocal cross was also made, viz, short Notch female by male of selected stock, with results in F_1 as follows:

Notch (atavistic).	Normal ♀.	Normal ♂.
13.....	28	19
13.....	9	18
23.....	28	29

It is evident from the first cross that the gene for short Notch in the sex chromosome of the male does not carry a dominant allelomorph for short Notch, and not even one that when heterozygous makes the Notch any shorter than in the atavistic flies. It is evident from the second reciprocal cross that the modifying gene for short Notch carried by the other sex chromosome, viz, the one that carries Notch also, is likewise not a dominant or even a modifier when in heterozygotic condition. Both crosses show that the selected stock is free from modifiers for making Notch shorter than the atavistic Notch.

In four cases the cross between short Notch and black pink bent was carried to the F_2 generation (instead of back-crossing as above) with the following results:

Notch.		Total all flies.
Short.	Intermediate.	
10	3	59
21	0	94
74	1	143

In this case the F_1 male gets his single X chromosome from his mother, viz, an X with the shortening factor. His sisters have one Notch X chromosome carrying the shortener and another from the bent father without the shortening factor. Hence the expectation in F_2 is that all the Notch flies should be short-Notch except for crossover cases, where the gene for short-Notch carried into the X is derived from

the bent father. The results agree with the expectation. The few intermediate-Notch flies may be such crossovers or more probably fluctuations of the short type.

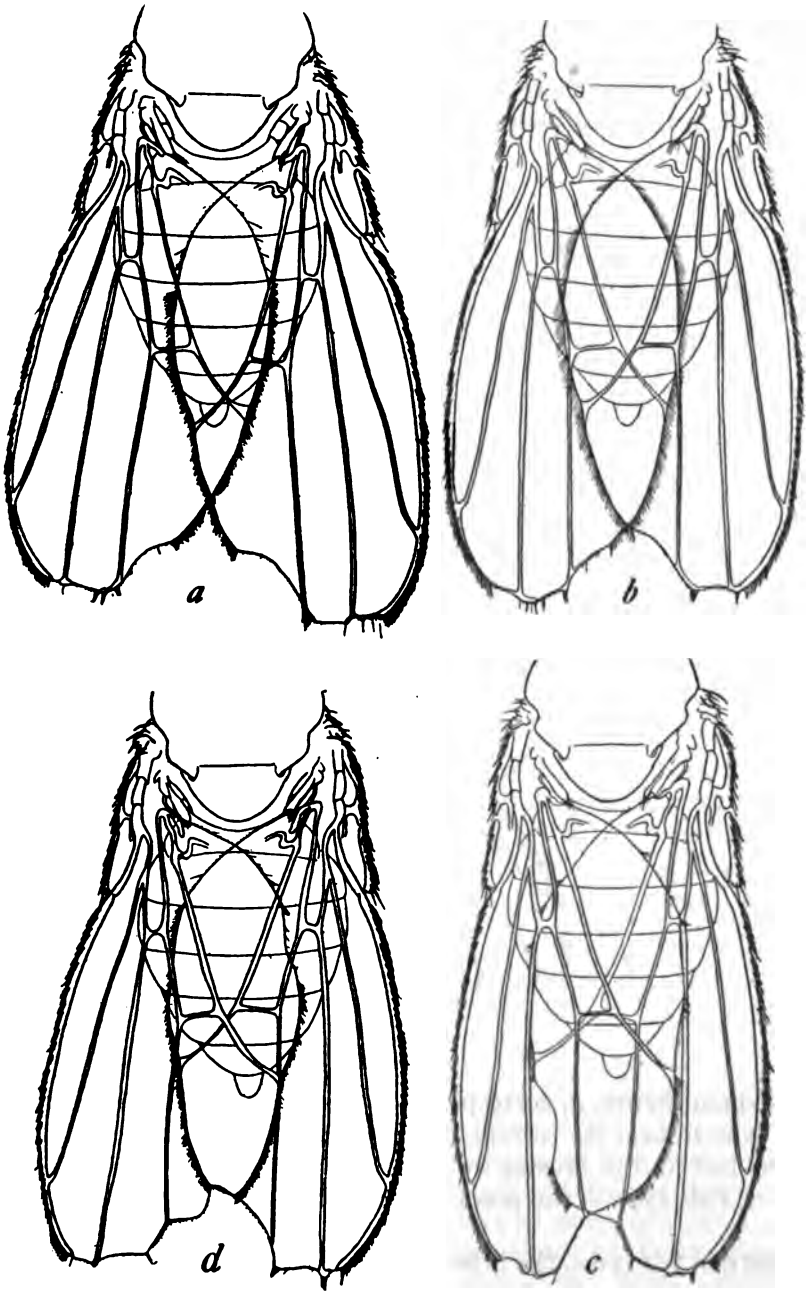


FIG. 97.

CLASSIFICATION OF TYPES OF NOTCH.

In the preceding crosses between short Notch and other races, I was handicapped by the difficulty of giving a more exact classification of the types called atavistic, intermediate, and "short" Notch. In order to get a more objective classification three characteristic flies were picked out from the short-Notch stock (that had been inbred for at least 25 generations, although not in pairs), and drawn (fig. 98, *a*, *b*, *c*).

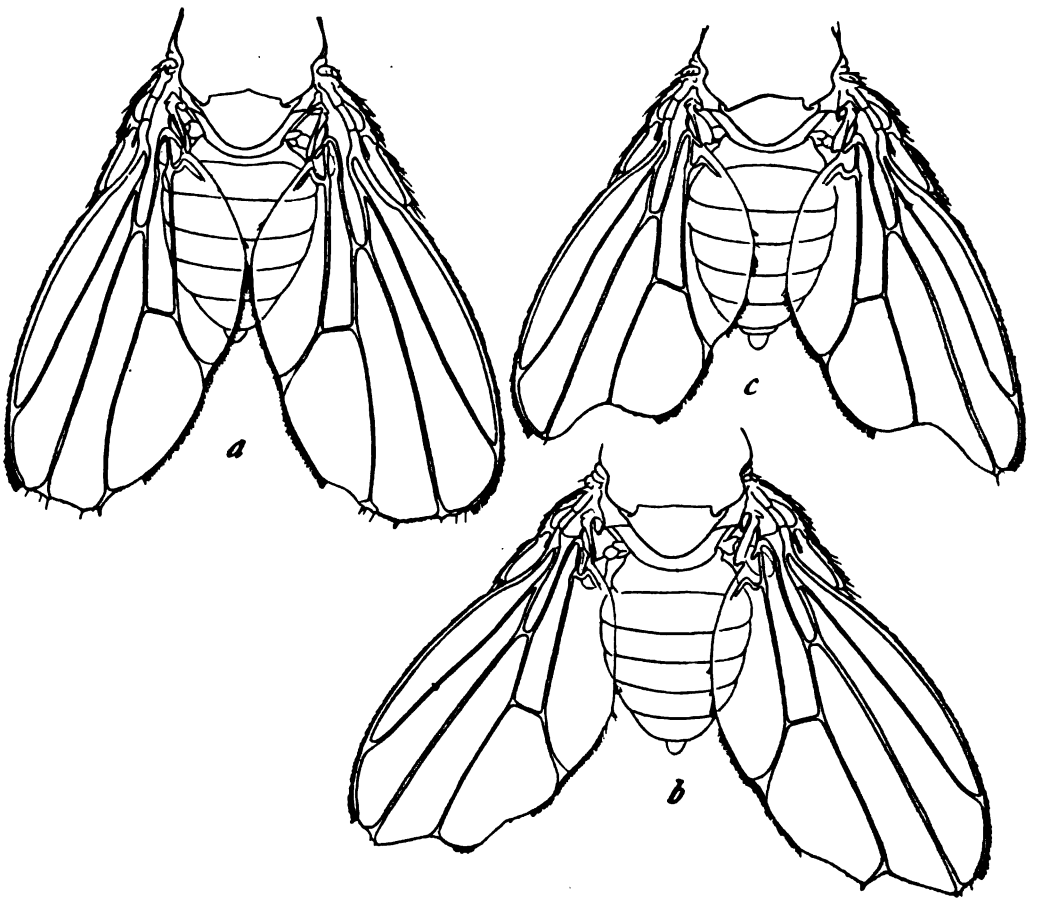


FIG. 98.

The left-hand figure, *a*, corresponds to the type called intermediate in hybrid Notch flies; the second, *b*, is a common type somewhat shorter than the last and in crosses would ordinarily be placed with the next type, *c*. This type is the predominating type in "good" short-Notch stock.

In contrast to type *a*, the type called atavistic is shown in figure 94, *b*. These two types overlap, but in a given case one or the other type is found in the great majority of individuals.

A census of the short-Notch stock taken at the same time that the two following records were made (April 1918), and under the same conditions, gives for these mass-cultures the results shown in table 14.

TABLE 14.—*Short-Notch stock for control.* (*Sh. Notch* ♀ *by eosin* ♂.)

Bottle.	Eosin ♂.	Eosin ♀.	Normal ♀.	Atavistic.	*a*.	*a*+.	*b*.	*b*+.	*c*.
1.....	20	29	.....	.....	.....	.....	3	3	13
2.....	16	13	1	.....	.....	.....	.....	1	10
3.....	96	110	3	1	3	7	10	14	41
Total....	132	152	4	1	3	7	13	18	64

Here, as is usual, an eosin-eyed father had been bred to a red-eyed short-Notch mother. The expected classes (non-crossovers and crossovers) are given in the first three columns, while the red-eyed Notch females are put into five classes that follow, viz, type atavistic; type *a*, "intermediate"; type *a*+ "intermediate" standing between *a* and *b*; type *b* "common" short Notch; type *b*+ standing between *b* and *c*; and type *c* "short Notch," the modal class.

A cross between sisters of the mothers of the above stock (short Notch) and wild males was made. The F_1 results are shown in table 15, in which the same classification of the F_1 Notch females as that just given for the stock control was made.

TABLE 15.—*Short Notch* ♀ *by wild* ♂.

Bottle.	Normal ♀.	Normal ♂.	Eosin ♂.	Atavistic.	At. 1 wing.	*a*.	*a*+.	*b*.	*b*+.	*c*.
1.....	10	.....	2	7	1	.....	.....	.....	.....	.....
2.....	9	.....	4	1	.....	3	1	4	.....	.....
3.....	42	2	47	32	4	4	1	.....	.....	.....
4.....	13	.....	18	.....	.....	1	1	6	4	5
5.....	23	1	18	7	1	.....	1	3	1	.....
6.....	3	.....	5	.....	.....	2	1	1	1	.....
7.....	48	.....	30	23	1	5	2	2	.....	.....
8.....	34	1	30	20	.....	5	4	.....	.....	.....
9.....	23	.....	12	3	.....	11	2	3	2	.....
10.....	47	.....	48	29	.....	8	3	5	2	.....
11.....	18	.....	14	1	.....	3	2	3	.....	.....
12.....	5	.....	9	.....	.....	.....	4	1	1	1
13.....	17	.....	21	3	.....	3	3	4	3	2
14.....	26	2	22	18	.....	1	5	1	.....	.....
15.....	37	.....	11	20	.....	4	2	.....	.....	.....
Total.	355	6	291	164	7	40	32	33	14	8

Table 15 shows the wide variability of F_1. The extreme plus variants, *i. e.*, the extreme short Notch class, are owing to three bottles where non-virginity of the female would be the simplest solution were it not that only 12-hour females were used, which, while still leaving open

some question as to the virginity of the mother, yet makes that interpretation unlikely. It seems to me more probable that in these three cases the father carried a modifier for Notch. Excluding, likewise No. 9 on the same grounds, it is evident from this table that the atavistic types predominate.

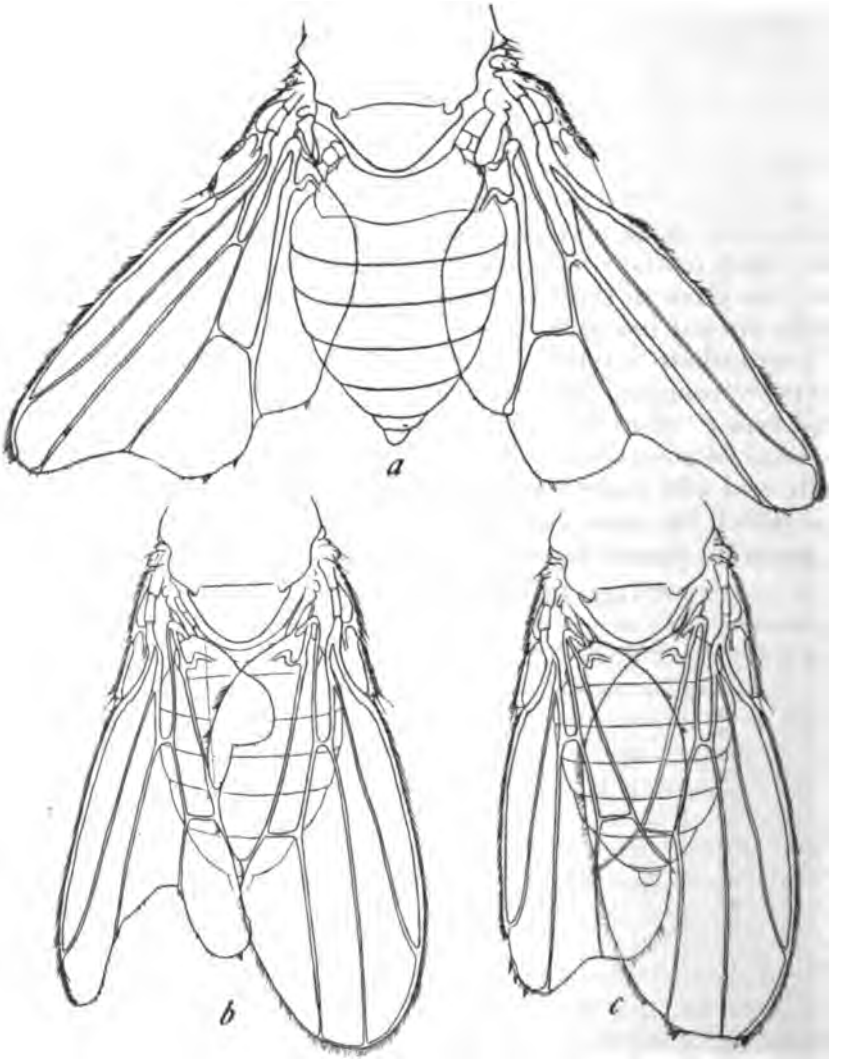


FIG. 99.

As I stated the yellow prune forked stock crossed to short Notch had given in F_1 so many intermediate flies that the experiment undertaken, to locate the short-Notch factor in the sex chromosome had to be abandoned. A year later the forked flies had disappeared from this

stock, so that only yellow prune flies could be utilized to again test this cross. The results confirmed the earlier ones, as seen in table 16.

The flies in all but one culture (No. 4) give nearly the same results as do the short Notch short females bred to their own stock males. Either the same factor for short Notch is carried by yellow prune that is present in the short Notch stock, or else some other factor that has a closely similar effect. As yet I have not put this question to a test. Culture No. 4 gave such a different result from the other five that it is almost certain that the "short" modifier was absent in this case.

TABLE 16.—*Short Notch* ♀ *by yellow prune* ♂.

Bottle.	Normal ♀.	Normal ♂.	Eosin ♂.	Eosin ♀.	Yellow-prune ♂.	Ata-vistic.	At. 1 wing.	*A.*	A+.	*B.*	*B*+.	*C.*
1.....	15	.....	22	.....	3	.....	.....	....	3	4	5	7
2.....	33	.....	41	.....	.....	.....	.....	4	....	5	10	11
3.....	1	.....	2	.....	.....	.....	.....	....	....	....	1	2
4.....	114	2	109	3	7	4	.....	26	3	32	27	11
5.....	24	.....	28	.....	.....	.....	.....	1	....	3	8	16
6.....	23	.....	16	.....	1	.....	.....	....	....	3	7	18
Total..	210	2	218	3	11	4	.....	31	6	47	68	65

ABERRANT NOTCH WINGS.

In the course of the selection experiment a few individuals appeared in which the wings showed an extreme condition of Notch. That these rare cases were due to some abnormal condition that influenced the development of the wing is shown by the fact that in most cases only one wing was affected, as shown in the three cases drawn in figure 99, *a*, *b*, *c*, and by the fact that when these were bred the offspring were of the usual Notch type. When both wings were affected (fig. 90, *a*) the flies were usually sterile. Possibly the results were due to somatic mutation, but this is not very probable. Two of the three flies had one normal wing, but the eye-color showed that the flies were genetically Notch. Similar modifications were seen in the eosin ruby sisters of these flies that did not contain the Notch-bearing gene.

DEFORMED EYES.

From time to time flies appear in the Notch stock in which one or both eyes are reduced (fig. 100), sometimes to mere specks. All attempts to breed such a type have been futile (although the males at least are fertile) and all attempts to cause them to appear in larger numbers by alterations in the environment (heat, cold, acidity, moisture) have failed. The remnant of the eye arouses a suspicion that the eye has been injured either by the larvæ in feeding or else by shaking the bottle containing the pupæ. We meet, not rarely in other stocks,

with injured eyes that have developed into smaller eyes, *i. e.*, dwarf whole eyes. Three conditions make this interpretation improbable. In the first place, the reduced eyes are often identically the same on the two sides, which would scarcely be expected if due to accidental puncture by a larva. In the second place, several individuals with reduced eyes often appear at the same time, while for long periods none at all are present. Some unknown environmental factor would seem the most probable explanation, especially when the offspring of Notch individuals do not repeat the eye condition. Probably some lethal combination may be involved. In the third place, the individuals which are not Notch have never shown this modification of the eyes. At the time when the deformed eye was most frequent (winter of 1917) records were kept of its frequency in mass-cultures (usually F_2 parents and F_2 offspring).

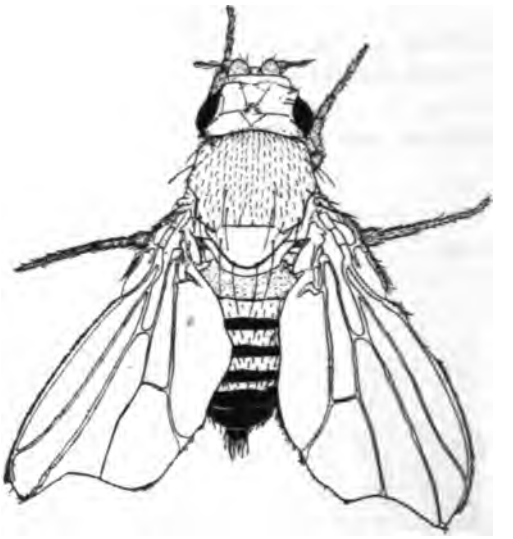


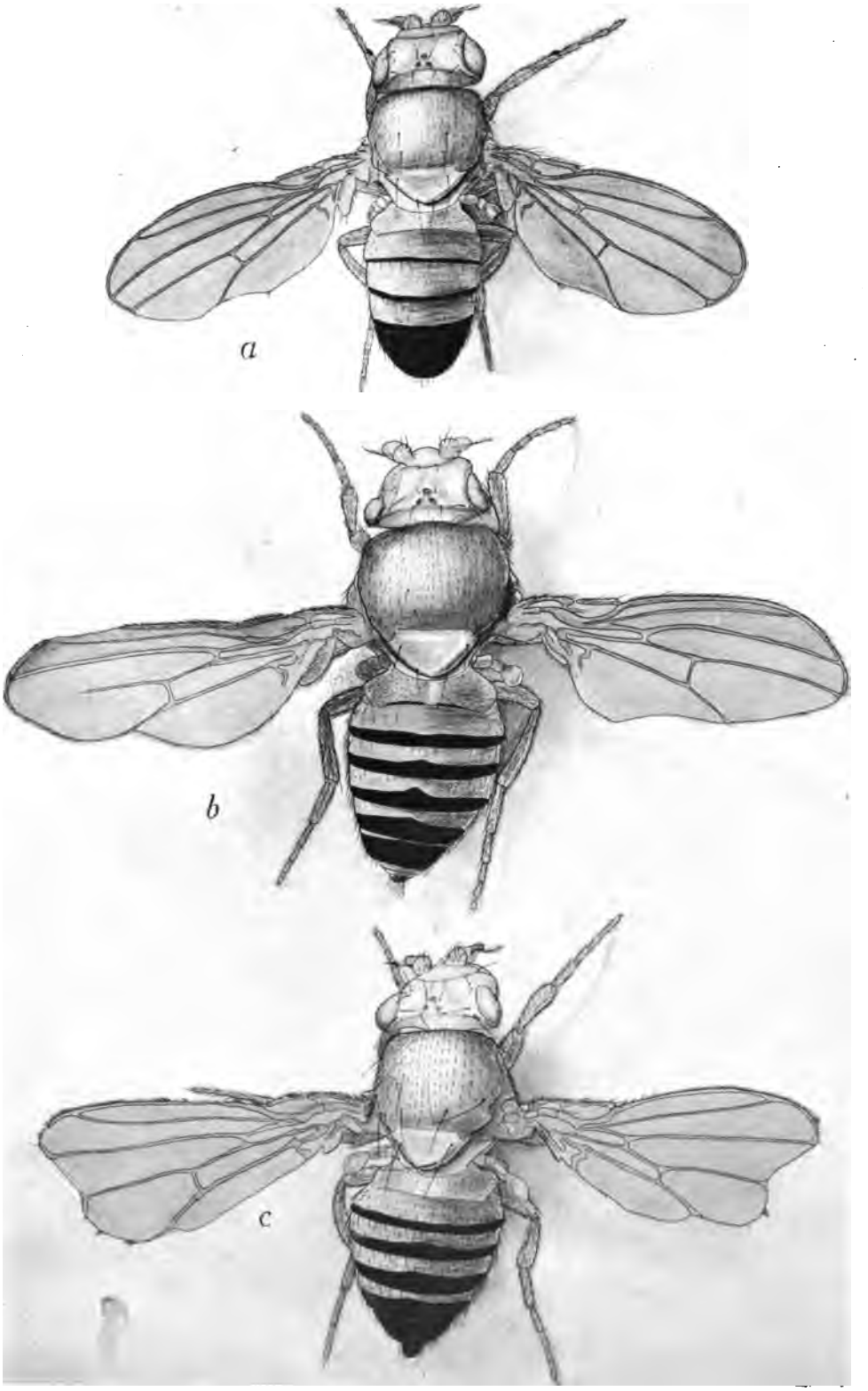
FIG. 100.

Gen.	Notch.	Deformed.	Red notch ♀.	Red deformed ♀.	Eosin ♀.	Eosin ♂.
F_3.....	1913	18	.....	.....	.....	.....
F_3.....	2475	20	.....	.....	.....	.....
F—.....	36980	10	.....	.....	.....	.....
F_4.....	.....	.....	12	1	65	70
F_5.....	.....	.....	72	10	78	83
F_5.....	.....	.....	26	13	85	52
F_6.....	.....	.....	20	.....	38	27
F_6.....	.....	.....	14	.....	79	71
F_7.....	.....	.....	24	0	18	25
F_7.....	.....	.....	19	8	74	59
F_7.....	.....	.....	30	2	29	33
F_7.....	.....	.....	22	0	37	31

In the culture F_5 there were 13 Notch with deformed eyes. These were all bred together with their normal brothers and sisters with eosin eyes and gave:

F_6: *Notch* ♀, 56; *deformed* ♀, 4; *eosin* ♀, 65; *eosin* ♂, 48.

The offspring of this line, part of which are included in the F_7 count in the preceding table, gave occasionally deformed-eyed flies, but not more frequently than sister cultures.


a
b
c

LITTLE EYES.

There appeared in the SS AAA 3346262 generation of the selected stock some mutant flies that had not only the wings something like those of Notch, but the eye was also of ten reduced (plate 12, *a*, *b*, *c*). Since the latter condition had been also found occasionally in short stock, the occurrence here of this new type, called little eyes, suggested the possibility that a new allelomorph of Notch had appeared. The sequel shows the futility of any such judgment in regard to the gene based on the appearance of the character. The new mutant proved to be so weak, so inviable, and so infertile that almost nothing could be done with it. It could, in fact, only be kept in existence by large mass-cultures of flies known to contain the genes. The females never bred, although many attempts were made to breed them. A few males mated to ruby females gave offspring, and these F_1 flies gave, along with many normal offspring, a few small-eyed flies of both sexes. The numbers were very small, but as both males and females were present, the result shows that the character is not sex-linked and therefore that it can not be an allelomorph of Notch. The location of the gene in its chromosome was not made out because the stock died. It will be noted that two of the females figured have Notch-like wings, while the other female and the male have rounded wings. It is probable that the two females really had the Notch gene, since the mutant arose in the stock, but other females were not Notch, as shown here and as frequently observed in later cultures. There is no evidence that any males were notched, although the beading might closely resemble notching. Great variability of the character was observed—in fact, some individuals could be detected only by the very slightly smaller eye or a tendency for the wings to spread out.

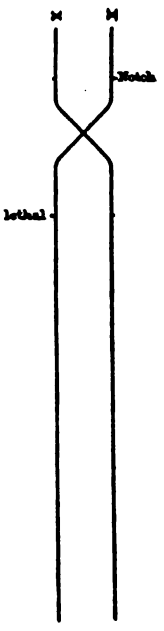


FIG. 101.

HIGH SEX-RATIOS CAUSED BY LETHALS.

Notch is a recessive lethal, and if by chance another lethal had been present in the X chromosome from the father of such a female, all of her sons would die except the occasional son due to crossing-over between the lethal factors. For instance, if the Notch gene has the location shown in figure 101 and another lethal factor in the other chromosome located as shown in the same figure, then either chromosome that goes into an egg that is later fertilized by a Y-bearing spermatozoon will die, but by crossing-over between the Notch and the lethal loci there will be produced one chromosome bearing two lethals, and another with their normal allelo-

morphs; the former will be expected to kill any male that gets it, the latter should give normal males. Hence a few males are expected under these circumstances—the number depending on the "distance" apart of the lethals involved. Two cases in which lethals appeared are given below:

	Notch ♀.	Normal ♀.	Eosin ruby ♀.	Eosin ruby ♂.	Eosin ♂.	Ratio.
X 667763...	5+6	36	29	1	.....	76 ♀ to 1 ♂.
SSO 1122...	44 4	71	.....	9	1	119 ♀ to 10 ♂.

The question of origin of the new lethal that appeared is not without interest. All of the eggs must contain it in the X chromosome allelomorphic to the one carrying Notch; hence it must have arisen in a single primordial cell from which all the cells of the ovary have come, or else it must have been present in the single spermatozoon that fertilized the egg from which the female in question developed. Since the new lethal is here contained in the eosin-ruby-bearing chromosome it is shown to have come from the male, and it seems probable here (although not explicitly shown since the behavior of the sisters is not recorded in the table), from a single one of his spermatozoa, viz, the one that fertilized the female under discussion. If subsequent work proves that when this kind of lethal arises the sisters of the lethal-bearing female are not lethal-bearing, it follows that the mutation took place in the last stages of the formation of the spermatozoon and perhaps at the time of maturation (which would give two such sperm), or even later after the sperm itself is formed.

The complete proof that the high sex-ratios here found are due to a lethal can only be established by breeding the daughters and by showing, as has been done in other such cases of high sex-ratios, that two lethals were present. As this point has been sufficiently established in other instances, it was not thought worth while to test it out here.

OTHER CHARACTERS THAT LOOK SOMETHING LIKE NOTCH.

In the course of the selection experiment a number of other characters have come up, and, since they involved the ends of the wings, might be taken by a neophyte as modifications of Notch or even perhaps as "caused" by the selection of Notch. Two points may be noticed here: first, that three of these mutations at least were in the direction opposite to the direction of selection, and second, that they might act as modifiers of the character selected, even although they happened to be mutations already known.

Cut is a mutant with outer and inner edge of wing cut off, leaving a pointed end (fig. 102). It is a well-recognized character and appeared in a male in one of the selected cultures, viz, SS AAA 874626114.

Truncate is a mutant that frequently appears in our cultures. It has also appeared in the selection experiments. The ends of the wings are cut off squarely. As it is dominant, especially in certain stocks, it is likely that it would much effect the character of the Notch when it occurred with it. Truncate appeared several times in the course of the experiment. The character of the truncate Notch flies is shown in figure 103, *a*, *b*, *c*, *d*.

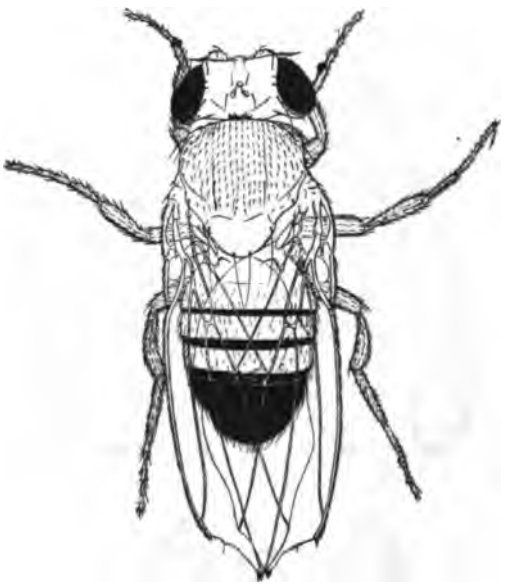


FIG. 102.

Beaded has appeared several times in the course of the work (fig. 104), and while no tests have been made to establish its relation to stock beaded, it is not unlikely that it is sometimes the same. Since beaded often affects the ends of the wings, and since Notch itself often has a defective outer margin to the wing, the similarity of the two stocks is in some flies very striking. But the common beaded is not sex-linked.

A stock which, when crossed to vestigal, produces flies many of which have a Notch on the end of the wings has been isolated by Dr. C. B. Bridges. It has no relation to Notch and appears in both sexes. (See "Nick," page 273, Part II.)

On several occasions males (also females) have been found in which a little piece is cut out from the end of one or from both wings. (fig. 105 *a*, *b*, *c*). Superficially one gets the impression that the Notch character has appeared in a male. These males have been bred, and have never transmitted the character, so that there can be no doubt that the variation has nothing whatever to do with Notch and is possibly only a somatic defect, or more probably is a multiple-factor character. The only way, in fact, that Notch might appear in a male would be through somatic segregation in a female embryo of such a kind that the Notch-bearing chromosome became dislocated and carried to the anlage of the wing, leaving the other chromosome to produce a male. Such a result has not been observed and it would be difficult to establish the case if it really occurred. The sex-linked mutant "serrate" that was present in the Star Dichæte stock is also a good mimic of Notch.

GYNANDROMORPH; NOTCH EOSIN RUBY.

In generation SS 11240521114 of selected Notch, an individual was found that was female, red-eyed and Notch wing on one side and male, eosin ruby-eyed and normal wing on the other side of the body. The mother of this fly had an X chromosome containing the gene for

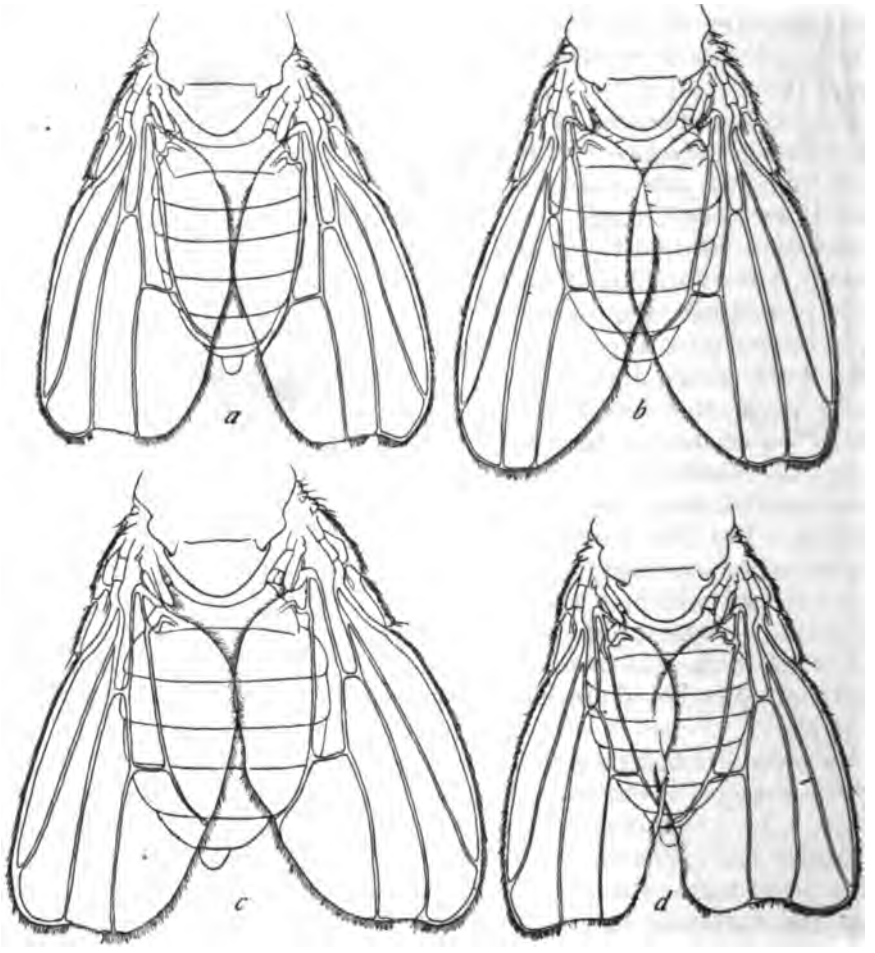


FIG. 103.

Notch and the normal allelomorphs of eosin and ruby (viz, red), and another X-chromosome containing the genes for eosin and ruby eye-colors. All of the characters for which these genes stand appear in this individual. An egg containing the Notch-bearing X must have been fertilized by a sperm containing the eosin ruby genes. At some time in the early history of one of the segmentation divisions of a nucleus of this egg, the eosin ruby-bearing X chromosome must have divided normally, while at the same time one daughter chromosome of the

other X (the Notch-bearing chromosome) must have lagged behind at some division, with the result that one cell got both X's and the other cell only one X.

In consequence of such a process of chromatin elimination we expect one part of the individual to be male as well as eosin ruby and the

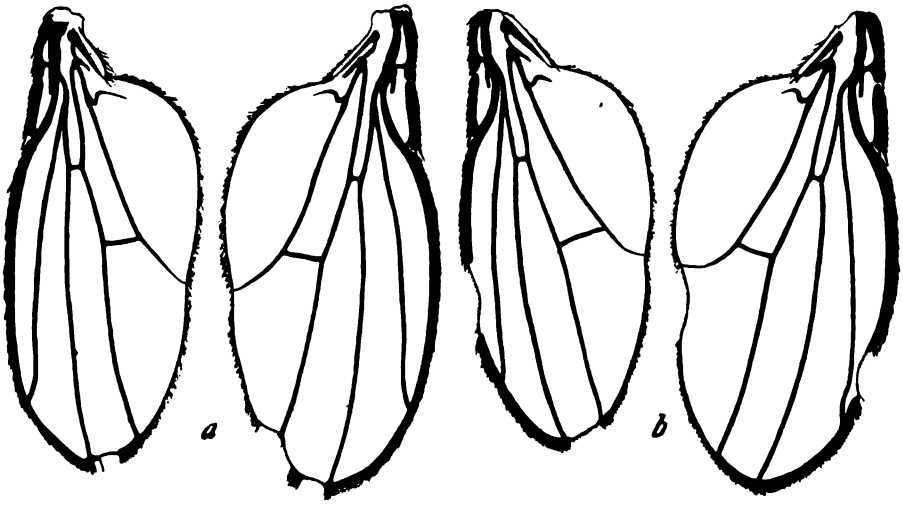


FIG. 104.

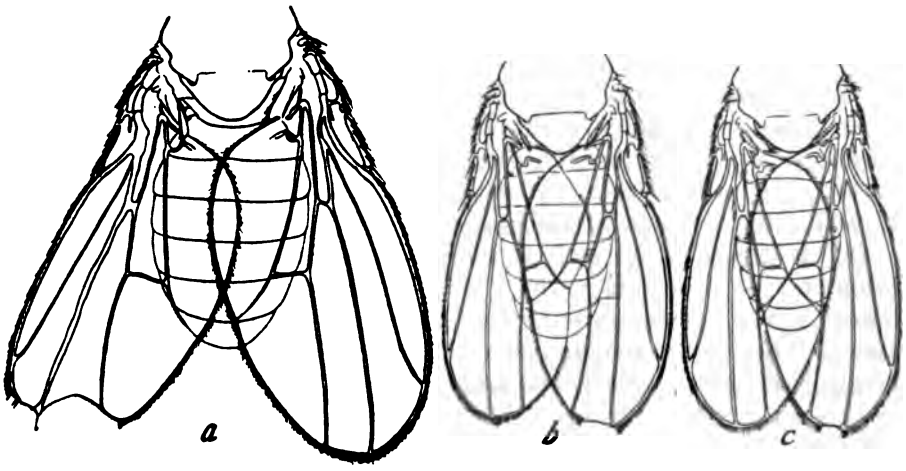


FIG. 105.

other part female, red-eyed and notched. An examination of Plate 4, figure 2 shows that the right side is female, as seen in the wings, the eye, and the foreleg (absence of sex-comb), and that the left side is male, as seen in the size of the wing, color of eye, and sex-comb. This gynandromorph is not, however, strictly bilateral, for the upper posterior corner of the light-colored eye is red, while the tip of the abdomen,

especially on the right side, is male. The genitalia (not shown here) are like those of the normal male. While, therefore, there is no such sharp line of division as is found in many *Drosophila* mosaics and gynandromorphs, yet the distinction between the characters in the different regions is sharp. There is nothing in the hypothesis of chromosomal elimination that requires that the critical division should occur so early that the nuclei that go to one half of the egg are separated from those that go to the other, or that even if it occurs at a very early division the separation of the two groups of nuclei need be exactly medial.

The critical evidence obtained in other *Drosophila* gynandromorphs proves that abnormality must have been due to chromosomal elimination rather than to other processes, such as those suggested earlier by Boveri (1883) and by myself (1905) to account for other gynandromorphs. The critical evidence rests on the presence in the two parents of a pair of genes in other than the sex chromosome. The same analysis can not be used in a case of this kind where only sex-linked characters are involved.

An examination of this case from the point of view of the two other hypotheses referred to above leads to the following analysis: Boveri's view calls for belated fertilization, so that the entering sperm unites with one only of the two first-division products of the egg-nucleus. Now, in this case we know that the egg-nucleus contained the genes for red-eyed and Notch, hence the products of such a division also contained these genes. If then to one of them the sperm-nucleus is added (bearing the eosin ruby genes) that half will give rise to female parts having the dominant character (red eyes and Notch wings), and the other first divisional product of the nucleus (haploid with one X), while expected to produce male parts perhaps, yet such male parts would have red eyes and Notch wings also. Clearly Boveri's view will not fit this case.

On my earlier view, gynandromorphs in insects may arise from supernumerary fertilizations. In this case we must suppose that two female producing sperms enter the egg, one fusing with the egg-nucleus and give rise to the female parts, the other developing separately and giving rise to the male parts, which would then have the eosin-ruby eye-color and normal wings. My own hypothesis fits the present case, but I think nevertheless that all such cases in *Drosophila* are more probably due to elimination because where critical evidence has been obtained it shows beyond doubt that the result was due to chromosomal elimination.

SUMMARY.

(1) Mass selection on a dominant character called Notch was carried out through 24 generations of *Drosophila melanogaster*, with the result that a change occurred in the direction of selection. Notch wing is caused by a dominant gene in the sex-chromosome. In addition to its dominance, the gene produces a recessive lethal effect, killing every male that carries the gene. Notch females are heterozygous for the Notch gene, *i. e.*, one X chromosome carries the gene for Notch, the other X chromosome its normal allelomorph. The latter saves the female from the lethal effect of the Notch gene. Since no Notch males exist, it is not possible to state whether the Notch gene would also be lethal in double dose in the female, but that such is almost certainly the case is shown by the absence of such females that might arise through equational nondisjunction, *i. e.*, by two Notch-bearing chromosomes remaining in an egg that was then fertilized by a Y sperm. Such a female, if she could be produced, would have no sons, and all of her daughters would be Notch (instead of half of them as usual). No such female appeared. The case of two females with high sex-ratios described in this paper are shown to be due to a lethal factor that had appeared in the "normal" X chromosome of the father of the female in question, etc.

(2) By a suitable method described in the text it is shown that the changes brought about by selection were due to the presence in the stock of a recessive modifying factor in the second chromosome. Notch females homozygous for this factor give the "selected group." Those heterozygous for it or lacking it altogether give the atavistic or original group.

(3) Since in every one of the 24 generations of this experiment the gene for Notch is in a heterozygous condition an extraordinarily favorable chance exists for contamination of the Notch genes, if such a thing is possible. Were it possible the results of the selection might be supposed to be due to an influence of the normal gene on the Notch gene. Mass selection was practiced in the same direction that such a supposition would lead to. That the result was not reached in this way is shown not only, as stated above, through the demonstration of the specific modifier involved, but also by out-crossing; for if at any time the selected Notch females (even those not showing any Notch at all) are bred to flies of almost any wild stock, the atavistic Notch is recovered in the first generation. Here, owing to the dominance of the character, one can obviate completely the difficulty that Castle met with when studying the influence of selection on a recessive character. Castle was obliged to out-cross his rats and then inbreed the F_1. The chance, unless guarded against scrupulously, of introducing new genes into the result is ever present under such conditions and does

not appear to have been avoided by Castle, hence his appeal to contamination of genes to help him out of an apparent contradiction. In the present case of *Drosophila* the experiment is of a kind to demonstrate clearly whether contamination had occurred or not, and the results clearly show that it did not occur, even under the unusually favorable opportunities that heterozygosis for 24 generations offered.

(4) A modification of the Notch character appeared several times in the course of the work. This variation, called short Notch (fig. 94, *b*,), is in the opposite direction from the selected type "produced by selection." By proper tests it is shown that this variation is due to another modifying gene situated in the X chromosome itself. When in homozygous condition the gene shortens and broadens the Notch wing, producing a greater amount of curvature at the end. This variant, too, can be brought back at any time to the original or atavistic type by breeding to wild flies.

(5) In the course of the work a number of other mutations occurred, some of which modified the wing in somewhat the same way as the Notch gene itself (nick and cut), others modified the wing as dominants (truncate), or in the homozygous condition (deformed eyes, etc.). Other modifications causing serrations or notchings on the end of the wings are known in *Drosophila;* the location of these genes in other chromosomes or at other levels than Notch in the X chromosome shows that they are different from Notch. Were it not possible, as it is in this case, to check up such modifications that resemble somewhat the character under selection, one might easily be led to entirely erroneous deductions.

(6) In the course of the experiment two females appeared with exceptional sex ratios, viz, 76 ♀ to 1 ♂ and 119 ♀ to 10 ♂. Their occurrence is undoubtedly due to the appearance of a lethal in the "normal" X chromosome of the Notch mother, because in several cases such changes in the sex-ratio in *Drosophila* have been shown to be due to such a situation. In consequence of two lethals in the mother, one in each X chromosome, every son will die, except for an occasional cross-over that will give rise to a normal son. That this result is not due to the production of a homozygous Notch female by non-disjunction is demonstrated by the kind of daughters produced, which were half normal, half Notch. All must have been Notch if the mother had been a double lethal Notch female (XXY).

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